



**MICROBially ENHANCED TRANSPORT OF
HYDROPHOBIC ORGANICS IN SOIL**

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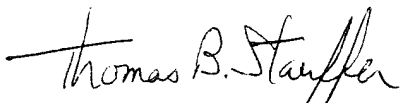
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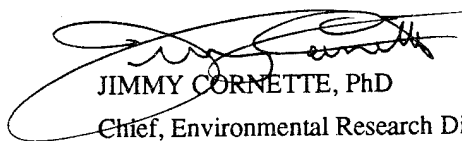
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13. ABSTRACT (Maximum 200 words) <p>Bacterial strains (many isolated from soil and subsurface environments) and their extracellular polymers were tested for their ability to enhance the transport of phenanthrene, a model PAH, in a low-carbon aquifer sand.</p> <p>All of the bacterial isolates tested passively sorbed phenanthrene, and most of the isolates reduced the distribution coefficient (K_d) for phenanthrene. Some isolates were also mobile in column experiments. The most mobile isolate significantly enhanced the transport of phenanthrene in the aquifer sand reducing its retardation coefficient by 25% at a cell concentration of $\sim 5 \times 10^7 \text{ mL}^{-1}$.</p> <p>Most (85%) of the extracellular polymers tested acted to decrease the phenanthrene distribution coefficient. Column experiments revealed a decrease in the retardation factor of phenanthrene by approximately 40% in the presence of an extracellular polymer produced by a gram (-) motile rod isolated from a coal tar waste site. This polymer did not inhibit the mineralization of phenanthrene and was not rapidly degraded by a mixed culture.</p> <p>The experimental results demonstrate that mobile bacteria may enhance the transports of PAHs in the subsurface. In addition, the combination of the ability of extracellular polymers to influence phenanthrene transport as well as their apparent persistence in the subsurface, suggest extracellular polymers have potential for enhancing PAH transport at groundwater remediation sites.</p>				
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PREFACE

The research described in this report was carried out at the School of Civil and Environmental Engineering, Cornell University in Ithaca, New York under contract No. F08635-90-C-0107 with the U.S. Air Force Engineering Services Center.

The assistance of AFESC senior scientist Dr. Thomas Stauffer is gratefully acknowledged. The authors thank C. Thomas (Section of Microbiology; Cornell Univ.) for providing bacterial isolates from an Electric Power Research Institute (EPRI) manufactured gas plant site, and for assistance with characterization of the isolates. P. Hatzinger (Soil, Crop, and Atmospheric Science; Cornell Univ.) provided a rhizosphere isolate (*Pseudomonas* A100), and P. Vandevivere (Soil, Crop, and Atmospheric Science; Cornell Univ.) provided bacterial isolates from the DOE Savannah River Plant site. The DOE isolates were originally obtained from the Subsurface Microbial Culture Collection at Florida State University (from Dr. D. Balkwill) which is supported by the DOE Subsurface Science Program (DOE grant DE-FG05-90ER61309). D. Czajka (Graduate Research Assistant, Environmental Engineering Program, Cornell Univ.) performed confirmatory polymer mineralization experiments. S. Best (Section of Microbiology; Cornell Univ.) provided electron microscopic analysis of isolate 9702M-4. K. Gardner (Graduate Research Assistant, School of Civil and Environmental Engineering; Cornell Univ.) assisted with the analysis of organic carbon on the aquifer sand.

The authors also thank Dr. Eugene Madsen and Prof. William Ghiorse (Section of Microbiology; Cornell Univ.), and Prof. Michael Shuler (School of Chemical Engineering; Cornell Univ.) for helpful comments and insights.

EXECUTIVE SUMMARY

A. OBJECTIVE:

The principal objective of this research was to test the effect of indigenous biotic components of soils and groundwaters, eg. bacteria and their extracellular polymers, on the sorption and transport of nonionic hydrophobic organic pollutants. The research sought to identify bacterial isolates and polymers that can enhance pollutant mobilization.

B. BACKGROUND:

Groundwater contamination is a common environmental problem at many U.S. Air Force installations. Some of the pollutants in question are hydrophobic, and sparingly soluble in water. Polynuclear aromatic hydrocarbons (PAHs) are exemplary compounds of this type and are known components of gasoline and jet fuel. Since some PAHs, such as benzo(a)pyrene, are known carcinogens and others are EPA priority pollutants, the presence of PAHs in groundwater constitutes a health hazard and their removal from contaminated sites is desirable. PAHs are strongly sorbed to soils, and consequently have low mobility. Therefore, the removal of hydrophobic aromatic compounds can dictate the time course for remediation of contaminated aquifers.

Hydrophobic contaminants in groundwater are frequently observed further down gradient and at higher concentrations than would be predicted based on their soil/water distribution coefficients. These results suggest that some process or mechanism may not have been considered in predicting pollutant sorption and transport. One such mechanism could be the presence of mobile colloids or dissolved macromolecules that may act as a "carrier" by sorbing the pollutant and transporting it through the porous matrix.

Bacteria are colloidal in size and indigenous to all soils, therefore they represent a natural means by which pollutant mobility may be enhanced. In addition, many soil bacteria produce extracellular polymers that can function as dissolved macromolecules that may bind hydrophobic pollutants such as PAHs, and therefore act to enhance the transport of such contaminants. Bacterial polymers naturally occur in groundwater.

For colloidal solids or dissolved macromolecules to function as carriers and increase hydrophobic pollutant mobility at least two conditions must be met: (1) the carrier must be able to bind the contaminant to an appreciable extent, and (2) the carrier

must have a relatively greater mobility than the contaminant molecule that it binds. This research evaluated the hypothesis that mobile bacteria and dissolved extracellular polymers of bacterial origin can meet these criteria. If the hypothesis is verified, then both mobile cells and dissolved polymers may be partially responsible for the enhanced transport that has been observed in the field.

If the factors resulting in facilitated transport of pollutants become known, risk assessment at spill sites will presumably become more accurate. If facilitated transport is not accounted for, high risk sites (i.e., those with conditions that result in high pollutant mobility) may be overlooked and contaminant transport underestimated.

A quantitative evaluation of carrier mediated transport may have practical applications beyond the explanation of observed contaminant behavior. Since transport of hydrophobic pollutants can dictate the time required for pump-and-treat remediation technologies, methods that increase the flushing rate of pollutants could be implemented to reduce the operating time of a facility. Modifications could either include *in situ* stimulation and release of biogenic carriers already present in the soil or could involve their addition to the infiltrating waters.

C. SCOPE:

This research was conducted using phenanthrene as the test sorbate and a low-carbon aquifer sand as the test sorbent. Phenanthrene is a representative polynuclear aromatic hydrocarbon, a known fuel component, and served as a model hydrophobic organic pollutant. Low-carbon sands are common in aquifer formations that are used to produce drinking water.

Over 20 bacterial isolates were evaluated in this research including well-characterized laboratory strains and many wild-type organisms isolated from soils. The effect on phenanthrene sorption and mobility was evaluated using suspensions of intact cells as well as the extracellular polymers produced by the test strains.

D. METHODOLOGY:

The methods employed in this research included:

1. use of batch isotherms to screen bacterial isolates and their extracellular polymers for their ability to decrease the sorption of phenanthrene onto a low-carbon aquifer sand,

2. use of batch sorption and miscible displacement experiments to test selected cell strains and polymers for their mobility in the low-carbon sand medium, and

3. use of miscible displacement experiments to test mobile polymers and bacterial isolates for their ability to enhance the transport of phenanthrene in a column of the low carbon sand medium.

In addition, selected polymers and cell strains were characterized to evaluate the relationship between their physical/chemical properties and their ability to influence the sorption of phenanthrene. Polymer size was determined by size exclusion chromatography and polymer functional groups were evaluated by infrared spectroscopy. The hydrophobicity of bacterial surfaces was determined by measurement of their distribution between an aqueous solution and a non-aqueous hydrocarbon phase.

Finally, experimental results for phenanthrene distribution and mobility in three component systems containing the phenanthrene sorbate, the low-carbon sand sorbent, and either dissolved extracellular polymers or suspended cells were compared to model calculations for the purpose of testing model validity.

E. TEST DESCRIPTION:

All experimental analyses were carried out in the Cornell University Environmental Engineering Laboratories. Batch sorption and miscible displacement studies were carried out using methods developed by the investigators. Experimental systems were constructed to minimize the interference with PAH behavior from hydrophobic synthetic polymers, such as Teflon®, that are common components of laboratory apparatus. Temperature control rooms and jacketed reactors were employed to ensure a constant test temperature of 25°C. Batch equilibrium and column studies were conducted using calcium sulfate as a model soil electrolyte and using sodium azide, a bacterial inhibitor, to prevent mineralization of the sorbate. Sterile techniques were used for transfer of bacterial strains and for their culture prior to harvesting cells or their extracellular polymers. Radiolabeled ¹⁴C-phenanthrene was used in all experiments and phenanthrene concentration was assayed by liquid scintillation counting.

F. RESULTS:

The bacterial cells and microbial polymers tested in batch systems were capable of influencing the sorption behavior of phenanthrene onto a low-carbon aquifer sand. Most (70%) of the bacterial isolates decreased the sorption of phenanthrene. While some extracellular polymers actually increased the amount of phenanthrene bound to the sand, most (85%) acted to decrease the amount sorbed.

Batch sorption experiments also revealed that selected bacterial isolates passively sorbed phenanthrene. Most of the bacterial isolates tested were able to move through packed columns of the low-carbon sand with negligible or slight retardation. Incomplete mass recoveries of the isolates tested indicated varying susceptibility to removal by filtration and/or slow desorption kinetics.

A polymer chosen for further study (produced by soil isolate 9702M-4) had a molecular weight of 500,000 daltons and was mobile in the low-carbon sand. Analysis by fluorescence quenching demonstrated that the polymer was an effective binding agent for phenanthrene.

The efficacy of bacterial extracellular polymers as macromolecular carriers for hydrophobic pollutants was confirmed by miscible displacement experiments in packed columns of the aquifer sand. Column experiments with the extracellular polymer from soil isolate 9702M-4 showed that, at a concentration of 100 mg C/L, the polymer decreased the overall retardation of phenanthrene by 39% (based on 80% recovery of input mass). Phenanthrene peaks appear earlier in the break through curve and with higher amplitudes and recoveries than the break through curve of phenanthrene in the absence of polymer.

The ability of an extracellular polymer to alter phenanthrene sorption onto the sand was not correlated with the functional groups obtained from infrared spectra or molecular size.

The presence of extracellular polymer acted to increase the overall mineralization rate of phenanthrene in an aqueous system without a sorbent and did not significantly alter the degradation rate of phenanthrene in an aqueous system with the low-carbon sand sorbent.

The efficacy of mobile bacterial cells as colloidal carriers for hydrophobic pollutants was confirmed by miscible displacement experiments in packed columns of the aquifer sand. Column experiments with soil isolate 9702A-2 and a methanotrophic

bacterium (OBBP) demonstrated that mobile bacterial cells could decrease the overall retardation of phenanthrene by $\approx 25\%$ at cell concentrations that were within the spectrum that naturally occur in aquifers.

The ability of bacteria to sorb phenanthrene and their mobility in the sand bore no apparent relationship to their hydrophobicity.

G. CONCLUSIONS:

The results obtained in this research conclusively demonstrate that both bacterial extracellular polymers and mobile bacterial cells can act as carriers to enhance the transport of a model polynuclear aromatic hydrocarbon (phenanthrene) in a low-carbon aquifer sand. Both extracellular polymers and mobile cells occur naturally in soil and aquifer systems and represent indigenous soil constituents that may be responsible for the facilitated transport of hydrophobic organic pollutants. The magnitude of carrier-induced transport is predicted to be a function of pollutant, soil, and carrier properties as well as the carrier concentration. In an aquifer sand, enhanced phenanthrene transport by mobile cells was observed at cell concentrations comparable to the ambient levels that are reported to occur in aquifer materials. Elevated concentrations of extracellular polymers were also shown to significantly enhanced phenanthrene transport. Since extracellular polymers may be readily produced in engineered reactor systems, the introduction of elevated polymer concentrations into contaminated soils appears to be feasible with current pump and treat remediation technology. Applications using both bacterial extracellular polymers and mobile bacterial cells warrant addition to the arsenal of technologies that are being developed to deal with groundwater contamination.

H. RECOMMENDATIONS:

This research was relatively basic in nature and demonstrated the influence of the presence of biogenic carriers on pollutant transport. Several alternative engineered methodologies are evident for application of the research results to the remediation of contaminated sites in the field. A major recommendation is that research be conducted to evaluate the feasibility of these alternatives. For example, addition of nutrient rich waters to a soil system may be considered for the purpose of stimulating *in-situ* production of extracellular polymers. However, these polymers may remain stationary, which in all likelihood would increase the sorption of hydrophobic pollutants onto the soil due to the increase in organic matter content, or the polymers could be released and result in facilitated transport of hydrophobic contaminants. Alternatively, bacterial polymers that are demonstrated to be very effective at mobilizing pollutants may be

grown in engineered reactor systems and applied with the infiltrating water in a pump-and treat remediation scheme. In this case, the susceptibility of added polymers to degradation by indigenous soil bacteria would be of concern. Polymer degradation would remove the added carrier from the soil system and could result in biofilm growth and clogging at the injection well. Laboratory experiments are needed to evaluate the feasibility of alternative engineered approaches that are designed to increase polymer concentrations and PAH transport in contaminated porous media. The extent to which microbes can be stimulated to produce polymers *in situ* and the mobility of the polymers that are produced must be evaluated as well as the behavior and fate of added polymers.

Since production of high polymer concentrations *in situ* would require an active microbial population, the effect of these cells in conjunction with polymer production must also be considered. The stimulation of cells that act as mobile colloids could facilitate pollutant transport in the groundwater but stationary cells may also sorb the pollutant. The utility of selectively stimulating certain bacterial populations (such as methanotrophic bacteria) merits additional consideration.

Concerted effort is warranted to determine if the facilitated transport of organic pollutants by biogenic agents can be coupled with enhanced mineralization rates. The bioavailability of sorbed organic pollutants may be limited and carriers that enhance pollutant concentration in the aqueous phase also have the potential to enhance mineralization rates. Extracellular polymers could therefore act to enhance both the transport and mineralization of sorbed hydrophobic pollutants. Testing of the coupled processes of PAH mineralization and transport in the presence of extracellular polymers is recommended. The bioavailability of cell-bound organic pollutants was not evaluated in this research; however it is deemed likely that desorption of a PAH that is mediated by cells may result in an enhanced rate of degradation. Testing of the coupled processes of PAH mineralization and transport in the presence of mobile cells is also recommended.

TABLE OF CONTENTS

	<u>Page</u>
Section I Introduction	1
A. Objective	1
B. Background	1
1. Sorption of Hydrophobic Compounds in Porous Media and Retardation of Transport	3
2. Microbial Polymers as Carriers	11
3. Mobile Cells as Carriers	13
4. The Effect of a Carrier on Pollutant Mineralization	14
Section II Experimental Methods and Materials	16
A. Reagents and Chemicals	16
1. Sorbate	16
2. Sorbent	18
3. Bacterial Polymers	19
B. Isotherm Experiments	23
1. Partitioning Between Phenanthrene and Sand	23
2. Partitioning Between Sand and Polymer	24
3. Partitioning Between Phenanthrene and Polymer	24
C. Characterization of Polymers	25
1. Infrared Spectra of Polymers	25
2. Size Exclusion Chromatography	25
D. Column Experiments	25
E. Phenanthrene Mineralization	30
F. Experiments with Bacterial Isolates	31
1. Culture Characterization	33
2. Whole Cell Phenanthrene Sorption Isotherms	35
3. Bacterial Cell Mobility in Porous Media	36
4. Column Studies with Phenanthrene in the Presence and Absence of Cells	37
Section III Results	39
A. Isotherm Data Analysis	39
B. Sorption of Phenanthrene	40
1. In the Absence of Polymer	40
2. In the Presence of Polymer	41

C.	Sorption Between Polymer and Sand	44
D.	Sorption Between Phenanthrene and Extracellular Polymer . .	44
	1. Data Analysis	44
	2. Results of Fluorescence Quenching Method	45
E.	Model Results	46
F.	Results of Column Studies	47
	1. Data Analysis	47
	2. Chloride Breakthrough	47
	3. Polymer Breakthrough	49
	4. Phenanthrene Breakthrough in the Absence of Polymer	50
	5. Phenanthrene Breakthrough in the Presence of Polymer	51
	6. Comparison with Model Calculations	52
	7. CXTFIT Calculations	53
G.	Extracellular Polymer Characterization	56
	1. Size Exclusion Chromatography	56
	2. IR Spectroscopy	57
	3. Polymer Production	59
H.	Phenanthrene Mineralization	60
	1. Mineralization in an Aqueous System without Sorbent	60
	2. Mineralization in an Aqueous System with Sorbent . .	61
I.	Batch Experiments with Whole Bacterial Cells	62
	1. Phenanthrene Sorption to Bacterial Isolates	62
J.	Sorption Experiments with Exopolymer from Methanotrophic Bacteria	66
K.	Bacterial Cell Mobility	67
L.	Phenanthrene BTCs with and without a Bacterial Carrier . .	72
M.	Model Predictions	76
N.	Culture Characterization	79
	1. Bacterial Cell Hydrophobicity	79
	2. Characteristics of Isolates 9702M-4 and 9702A-2 . .	81
	3. Soluble Methane Monooxygenase Activity of Strain OB3b	82
Section IV	Conclusions and Recommendations	84
Appendix	88
Glossary of Symbols	90
References	92

LIST OF FIGURES

	<u>Page</u>
Figure 1	A conceptual model for pollutant distribution in a three-compartment (aqueous, dissolved macromolecular carrier, soil) system. 6
Figure 2	A conceptual model for pollutant distribution in a three-compartment (aqueous, colloidal bacterial carrier, soil) system. 8
Figure 3	Effect of the partition coefficient between the pollutant and the carrier on the retardation factor of a hydrophobic pollutant as a function of the ratio of the carrier partition coefficient with the pollutant to the carrier partition coefficient with the stationary phase, $r = K_d^{om}/K_{dom}^s$. [All calculations assume the sorbent and sorbate combination are comparable to phenanthrene and an aquifer sand, $K_d^s = 12.9$ and $\rho_b/n = 4.38$.] 9
Figure 4	Effect of the partition coefficient between the pollutant and the carrier on the retardation factor of a hydrophobic pollutant as a function of carrier concentration. [All calculations assume the sorbent and sorbate combination are comparable to phenanthrene and an aquifer sand, $K_d^s = 12.9$ and $\rho_b/n = 4.38$.] 10
Figure 5	Saturator device used for preparation of phenanthrene solutions. 18
Figure 6	Schematic of assembly used for miscible displacement experiments. 26
Figure 7	Custom column apparatus designed by Lion et al. to minimize hydrophobic fittings. 28
Figure 8	Phenanthrene sorption isotherm on aquifer sand with aqueous concentrations approaching the solubility limit. 41
Figure 9	Phenanthrene sorption isotherm on an aquifer sand in the presence and in the absence of 100 mg TOC/L of extracellular polymers produced by soil isolate 9702M-4. 42

Figure 10	Summary of the predicted effects on the retardation factor or phenanthrene in an aquifer sand in the presence of 100 mg/L TOC/L of the indicated microbial polymers.	43
Figure 11	Stern-Volmer plot for the partitioning of phenanthrene onto the extracellular polymer produced by soil isolate 9702M-4.	46
Figure 12	Column breakthrough of chloride and the least squares fit calculated from CXTFIT.	48
Figure 13	Breakthrough curve of extracellular polymer produced by soil isolate 9702M-4.	49
Figure 14	Phenanthrene breakthrough curves in the presence and in the absence of the extracellular polymer produced by soil isolate 9702M-4.	52
Figure 15	Typical phenanthrene BTC in the absence of polymer and comparison to a fit of the data to the two site/two region model generated using CXTFIT.	54
Figure 16	HPLC calibration curve used to estimate the molecular weight of microbial polymers	57
Figure 17	Production of extracellular polymer from bacterial isolate 9702M-4 (●) as a function of cell mass (○) and optical density of the culture broth (□)	59
Figure 18	Effect of dissolved extracellular polymer on the mineralization of dissolved phenanthrene (in the absence of a sorbent) by a mixed bacterial culture.	60
Figure 19	Effect of dissolved extracellular polymer on the mineralization of phenanthrene (in the presence of a sorbent) by a mixed bacterial culture.	61
Figure 20	Effect of cell concentration on the apparent K_d value for phenanthrene with an aquifer sand. (Results for soil isolate 9711A-2). [Model parameters: $A = 21.2$, $B = 35.0$, $RSS = 0.06$].	64

Figure 21	Break through curve of isolate A100.	68
Figure 22	Break through curve of isolate 9703A-1.	68
Figure 23	Break through curve of isolate 9701A-2.	69
Figure 24	Break through curve of isolate 9702A-2.	69
Figure 25	Break through curve of methanotrophic strain BG8.	70
Figure 26	Break through curve of methanotrophic strain OB3b.	70
Figure 27	Break through curve of methanotrophic strain OBBP.	71
Figure 28	Phenanthrene BTC with and without the presence of the subsurface isolate 9702A-2.	74
Figure 29	Phenanthrene BTC with and without the presence of the type II methanotroph OBBP.	75
Figure 30	Phenanthrene BTC with and without an eluent containing a cell suspension of isolate 9702A-2 ($57 \mu\text{g TOC mL}^{-1}$).	76
Figure 31	Comparison of predicted and observed reduction of K_d value . . for phenanthrene with the aquifer sand in the presence of various concentrations of isolate 9792A-2.	77
Figure 32	Comparison of predicted and observed reduction of K_d value . . for phenanthrene with the aquifer sand in the presence of isolate A100.	78
Figure 33	Comparison of predicted and observed reduction of K_d value . . for phenanthrene with the aquifer sand in the presence of strain BG8.	78
Figure 34	Comparison of predicted and observed reduction of K_d value . . for phenanthrene with the aquifer sand in the presence of strain OBBP.	79

Figure 35	Results of the MATH test showing the characteristic 80
	hydrophobivity of <i>A. calcoaceticus</i> ; the other isolates
	not showing cell-surface hydrophobicity were 9701A-1,
	9702A-2, 9709A-3, 9712M-3, A100, Nd9, and N1.

LIST OF TABLES

		<u>Page</u>
Table 1	Physical and Chemical Properties of Phenanthrene	17
Table 2	Aquifer Sand Characteristics	19
Table 3	Summary of Bacterial Isolates and their Sources	20
Table 4	EPS Growth Medium	21
Table 5	Growth Medium (mg/L) for Mineralization Studies	30
Table 6	Additional Bacterial Strains Used in Experiments with Whole Cells	32
Table 7	Results of Two-site/Two-region Model Fits for Phenanthrene Breakthrough Curves	55
Table 8	Elution Times for Standards and Selected Polymers	56
Table 9	Prominent Features of the IR Spectra for Selected Extracellular Polymers	58
Table 10	Partition Coefficients for Phenanthrene with Bacterial Cells	62
Table 11	Reduction of K_d Value for Phenanthrene with the Aquifer Sand	63
Table 12	Bacterial Isolates that most Effectively Decreased the Sorption of Phenanthrene onto an Aquifer Sand	65
Table 13	Effect of Extracellular Polymer from Methanotrophic Bacteria on the Distribution Coefficient (K_d) for Phenanthrene	66
Table 14	Distribution Coefficients for Phenanthrene with Methanotrophic Cells (K_d^{cell}), and for Methanotrophic Cells with an Aquifer Sand (K_{cell}^s).	71
Table 15	Rapid NFT Test Results for Isolates 9702M-4 and 9702A-2.	82

SECTION I

INTRODUCTION

A. OBJECTIVE

The principal objective of this research was to test the effect of indigenous biotic components of soils and groundwaters, eg. bacteria and their extracellular polymers, on the sorption and transport of nonionic hydrophobic organic pollutants. The research sought to identify bacterial isolates and polymers that can enhance pollutant mobilization.

B. BACKGROUND

Groundwater contamination is a common environmental problem at many U.S. Air Force installations. Some of the pollutants in question are hydrophobic, and sparingly soluble in water. Polynuclear aromatic hydrocarbons (PAHs) are exemplary compounds of this type and are known components of gasoline and jet fuel ^{1,2}. Since some PAHs, such as benzo(a)pyrene, are known carcinogens and others are EPA priority pollutants, the presence of PAHs in groundwater constitutes a health hazard and their removal from contaminated sites is desirable. PAHs are strongly sorbed to soils ³, and consequently have low mobility. Therefore, the removal of hydrophobic aromatic compounds can dictate the time course for remediation of contaminated aquifers.

The prediction of the transport of a nonionic organic compound in a porous medium such as soil or aquifer material requires an understanding of its distribution between the mobile (aqueous suspension) and stationary (solid) phases. Neglect of an important component or phase may lead to erroneous results. Several investigators have observed PAHs in groundwater further down gradient and at higher concentrations than would be predicted based on their soil/water distribution coefficients ^{4, 5, 6, 7, 8}. These results suggest some process or mechanism may not have been considered in predicting pollutant sorption and transport. One such mechanism could be the presence of mobile colloids or dissolved macromolecules that may act as a "carrier" by sorbing the hydrophobic pollutant and carrying it through the porous matrix. Such "facilitated transport" has been observed for hydrophobic compounds in the presence of humic materials as well as several exogenic and synthetic colloidal materials ^{9, 10, 11, 12}.

Bacteria are colloidal in size and indigenous to all soils, therefore they represent a natural means by which pollutant mobility may be enhanced. In addition, many soil bacteria produce extracellular polymers that can function as dissolved macromolecules that may bind hydrophobic pollutants such as PAHs, and therefore act to enhance the transport of such contaminants. Bacterial polymers naturally occur in groundwater ¹³. Both mobile cells and dissolved polymers, therefore, may be partially responsible for the enhanced transport that has been observed.

The phenomenon of size exclusion may also be involved in increased transport of hydrophobic compounds in soil and ground water systems. Both colloids and macromolecules are subject to the phenomena observed in size exclusion chromatography in which larger-sized substances are excluded from entry into small pores. In such a case, larger-size materials move with a higher velocity than an inert tracer, such as $^3\text{H}_2\text{O}$, that can enter all of the pore volume. Size exclusion in soils can enhance mobility of nonsorbing carriers relative to the nominal pore water velocity; this will result in a concomitant enhancement of the mobility of carrier-bound pollutants ¹⁴. Harvey et al. ¹⁵ have reported evidence for size exclusion of mobile bacteria in soil.

For colloidal solids or macromolecules to increase hydrophobic pollutant mobility at least two conditions must be met: (1) the carrier must be able to bind the contaminant to an appreciable extent, and (2) the carrier must have a relatively greater mobility than the contaminant molecule which it binds. In the case of bacteria, most research on colloidal-sized solids in groundwater has focused on factors related to mobility ^{16, 17}. Models of filtration processes ¹⁸ lead to predictions that particles $\approx 1 \mu\text{m}$ diameter (approximately the size of many bacteria) are likely to be mobile in porous media. Several environmental studies have demonstrated the mobility of both bacteria ^{15, 19}, and viruses ²⁰ in soil and ground water systems. The literature also contains a small number of reports on the sorption of hydrophobic compounds such as PAHs by microorganisms ^{21, 22}. These results suggest that cells can function as effective sorbents. There are relatively few studies, however, in which both cell mobility and pollutant sorption have been simultaneously considered. Lindqvist and Enfield have demonstrated enhanced transport of DDT in the presence of a *Bacillus* sp. ²³. Model calculations by these investigators indicated that a concentration of 10^6 cells/mL would increase the mobility of hydrophobic compounds with octanol-water partition coefficients $\geq 10^6$. Retarded, nonequilibrium movement of bacteria was observed in column transport studies carried out by these investigators but was not considered in their model ²³.

Studies of potential dissolved organic carriers have focused on their pollutant binding properties 7, 24, 25, 26, 27, 28, 29. In addition, a few investigations have helped to develop a qualitative understanding of carrier effects on pollutant mobility. Enhanced transport of hydrophobic pollutants has been observed in laboratory columns in the presence of humic material 30, and well-defined surrogates for dissolved and colloidal organics 11. Enfield et al. 10 demonstrated the enhanced transport of several pollutants, including PAHs, in laboratory soil columns using dextran as a macromolecular carrier. Both dextran and groundwater dissolved organic carbon (DOC) were shown to undergo apparent size exclusion from some pore space in the column, while a third potential carrier (humic acid) was retarded.

1. Sorption of Hydrophobic Compounds in Porous Media and Retardation of Transport

In the determination of the fate of a pollutant in the environment, it is useful to characterize the mechanisms involved in the distribution of the compound among the various phases and the reactions involved within each phase. Sorption of hydrophobic nonvolatile compounds from the aqueous phase to a solid phase is one of the most important of the mechanisms involved for this class of organic pollutants 3.

For nonpolar hydrophobic organic materials, a distribution between the aqueous and sorbed phase has been observed that can be closely correlated to the organic carbon content of the soil or sediment 3, 31, 32. Assumption of a partitioning process implies reversibility of the reaction 3 and a linear relationship between aqueous and sorbed concentrations of the solute 33, i.e.:

$$S = K_d C \quad (1)$$

where: S : mass of solute sorbed per unit mass of sorbent (mg/g),
 K_d : distribution or partition coefficient (mL/g),
 and C : aqueous concentration of solute (mg/mL).

If organic carbon is assumed to be the primary sorbent, the partition coefficient can be normalized according to the organic carbon content:

$$K_{oc} = K_d / f_{oc} \quad (2)$$

where: f_{oc} is the weight fraction of organic carbon in the sorbent.

K_{oc} values for many organic pollutants have been measured for several soils and sediments and have shown agreement within a factor of three ³⁴. K_{oc} values for organic pollutants are correlated with their octanol-water partition coefficients (K_{ow}) ³⁵ and may be readily estimated. With estimated values of K_{oc} and a given soil with known organic carbon content (f_{oc}), it is possible to estimate the sorption of a compound within a given soil/water system. However, not all types of soil organic matter have the same sorption characteristics ^{34, 36} and that, at low soil organic carbon content, mineral surfaces can become significant in the sorption of organic compounds ^{37, 38}. In addition, not all soil organic matter may take part in the sorption process ³.

The transport of an inert solute, such as chloride, through a porous homogeneous media can be modeled using the advection/dispersion equation, which may be derived from the conservation of mass. For transport in one dimension ³⁹:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v_w \frac{\partial C}{\partial x} \quad (3)$$

where C is the aqueous solute concentration,

x is the flow direction,

v_w is the interstitial flow velocity,

t is time,

and D is the hydrodynamic dispersion coefficient and consists of a molecular diffusion component and a mechanical dispersion component (due to shear flow in the porous matrix).

To include the sorption of the solute, the concentration sorbed to the solid phase, S , must be accounted for:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v_w \frac{\partial C}{\partial x} + \frac{\rho_b}{n} \frac{\partial S}{\partial t} \quad (4)$$

where: n is the porosity of the soil,

and ρ_b is the bulk density of the sorbent.

Assuming a linear distribution of pollutant between the aqueous and solid phases, the rate of change of sorbed concentration is proportional to the rate of change of the aqueous concentration by a factor equal to the distribution coefficient, K_d :

$$- K_d \frac{\partial C}{\partial t} = \frac{\partial S}{\partial t} \quad (5)$$

Given this relationship, the advection-dispersion equation [Equation (4)] becomes:

$$\frac{\partial C}{\partial t} \left(1 + \frac{\rho_b}{n} K_d\right) = D \frac{\partial^2 C}{\partial x^2} - v_w \frac{\partial C}{\partial x} \quad (6)$$

The factor obtained on the left side of Equation (6) is a constant for a given soil/pollutant system and is termed the retardation factor, R . Conceptually, the retardation factor defines the average velocity of water, v_w , relative to the average velocity of a solute, v_s , i.e.

$$R = \frac{v_w}{v_s} = 1 + \frac{\rho_b}{n} K_d \quad (7)$$

The more the solute is sorbed onto the soil matrix, the higher the retardation factor. The retardation factor of a compound is commonly used to predict contaminant mobility in a simple soil/water environment. However, as noted above, several investigators have observed elevated concentrations and enhanced transport of hydrophobic compounds that should theoretically have very limited mobility according to their soil/water partitioning properties 4, 5, 6, 7, 8. Such results suggest that there may be some consideration beyond simple sorption to the soil matrix that governs the transport and fate of hydrophobic pollutants.

Possible explanations for observations of enhanced transport may include fingering of flow in soil, nonuniform fluid transport through root and worm holes, or the presence of macropores. These are hydrodynamic processes that restrict contact of the solute with the sorbent. Another possibility that would apply even in uniform homogeneous porous media, is the presence of "carriers." A colloidal particle or dissolved macromolecule may have a significant binding capacity for the pollutant and may potentially carry it through the soil column relatively unretarded. Thus, in addition to the transport of the aqueous concentration of the pollutant that is taken into account by the simple two-compartment (sorbent/sorbate) transport model, a fraction of pollutant bound to mobile carrier would also be transported with the fluid flow. If carrier enhanced transport is considered, it becomes necessary to model the groundwater system as one consisting of three compartments; i.e.: the dissolved phase, the stationary particulate phase, and the mobile carrier phase 40. A conceptualization of the three component model is shown in Figure 1.

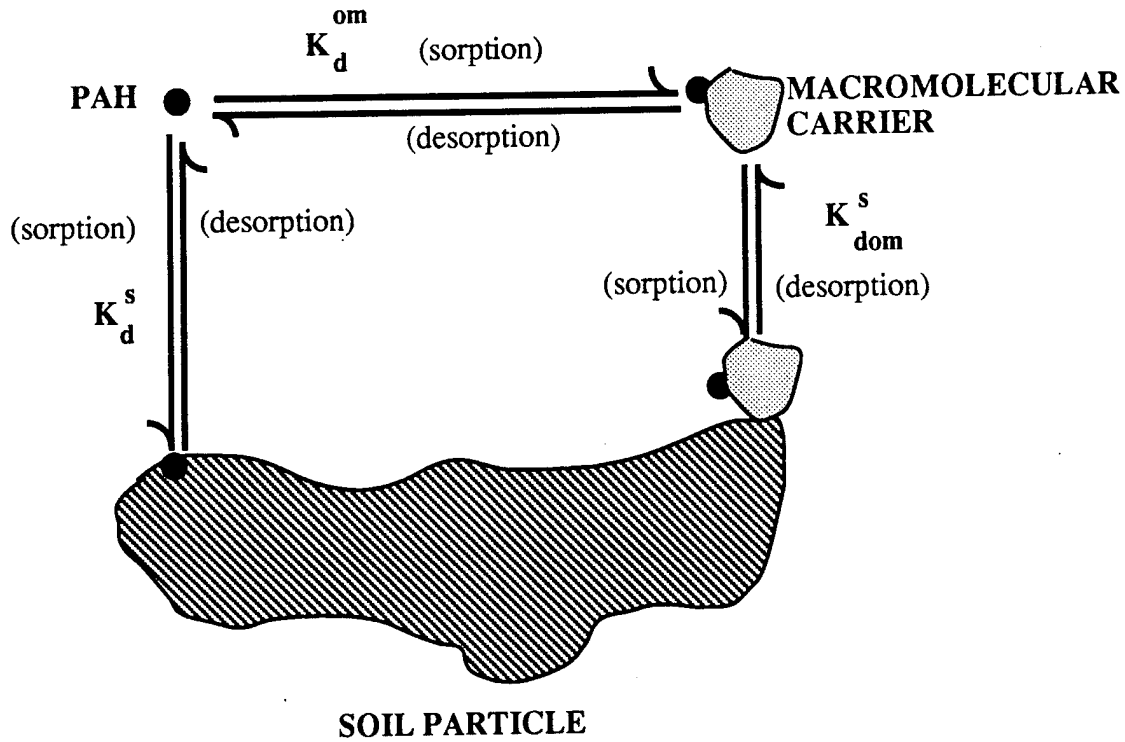


Figure 1. A conceptual model for pollutant distribution in a three-compartment (aqueous, dissolved macromolecular carrier, soil) system (after Magee et al. 12).

Using linear distribution coefficients between the compartments, Magee et al. 41) derived the following modified expression for the retardation factor:

$$R^* = \frac{(1 + K_d^{om} \text{DOM} + K_d^s \rho_b/n)}{1 + \frac{K_d^{om} \text{DOM}}{1 + K_{dom}^s \rho_b/n}} \quad (8)$$

where: DOM is the dissolved macromolecular carrier concentration,
 K_d^{om} is the partition coefficient between the carrier and the pollutant,
 K_d^s is the partition coefficient between the pollutant and the sorbent,
 and K_{dom}^s is the partition coefficient between the carrier and the sorbent.

If the average carrier undergoes size exclusion due to exclusion from micropores, i.e. $v_c > v_w$, the above equation becomes:

$$R^* = \frac{1 + K_d^{om} \text{DOM} + K_d^s \rho_b/n}{(v_c/v_w) K_d^{om} \text{DOM} + 1 + K_{dom}^s \rho_b/n} \quad (9)$$

where: v_c is the average velocity of the carrier-bound solute,
and v_w is the average velocity of the water.

A detailed derivation of the above relationships was given by Magee et al. 41, and is included as an Appendix to this report for the sake of completeness. Using sensitivity analyses, Magee et al. determined the model to be most sensitive to the carrier concentration (DOM in the above equations) and to the pollutant distribution coefficients to the soil (K_d^s) and to the carrier (K_d^{om}). Using the expression for pollutant retardation in the presence of a carrier that is given above [Equation (9)], it may be possible to predict more accurately the retardation of a hydrophobic compound in cases where carrier enhanced transport occurs.

As a first approximation, the three-compartment model may also be extended to experimental systems in which the carrier is a colloidal solid such as a bacterial cell. In this case, the sorption of colloid to the porous medium (or its removal by filtration) must be considered as a reversible process. At steady state, the ratio of the rates of colloid removal and release would result in a linear colloid "distribution coefficient" that would replace the sorption distribution coefficient for dissolved carrier in the model (see Figure 2). Under these circumstances, the mobility of bacterial cells in porous media becomes one of the criteria for bacterially facilitated transport of hydrophobic pollutants in the environment.

In order for a macromolecule such as an extracellular polymer or a colloid such as a bacterial cell to facilitate the transport of a hydrophobic compound, it must be able to bind the pollutant compound and be more mobile than the pollutant compound. This can be illustrated by application of the three component model for R^* . If the mechanisms responsible for the partitioning of the carrier to the pollutant and to the soil are similar, then the ratio of the respective partitioning coefficients can be treated as a constant, i.e.:

$$r = \frac{K_d^{om}}{K_{dom}^s} \text{ or } \frac{K_d^{cell}}{K_{cell}^s} = \text{constant.} \quad (10)$$

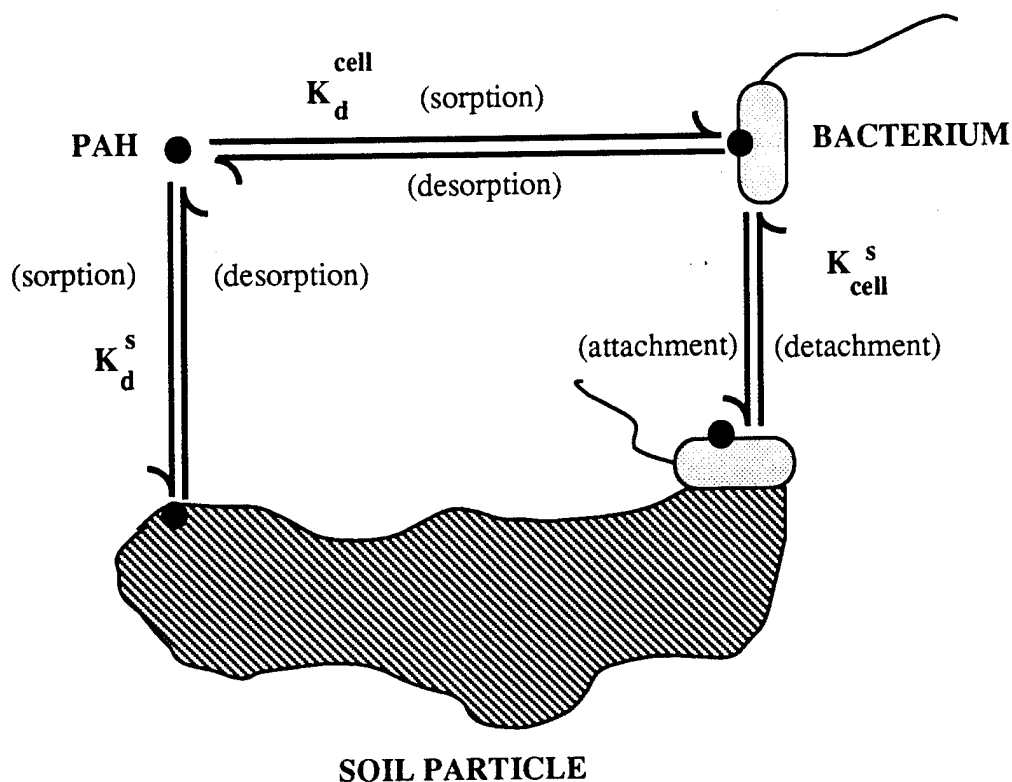


Figure 2. A conceptual model for pollutant distribution in a three-compartment (aqueous, colloidal bacterial carrier, soil) system (after Jenkins and Lion 42).

The above assumption might be observed if hydrophobic interactions govern both pollutant binding to the carrier and carrier binding to the soil. In such a case both K_d^{om} and K_{dom}^s would increase as the hydrophobicity of the carrier increased. For a fixed carrier concentration, the relationship for R^* predicts a distinct minimum in the retardation when it is plotted against the distribution coefficient of the pollutant to the carrier: K_d^{om} or K_d^{cell} (see Figure 3). At low values of K_d^{om} or K_d^{cell} , the carrier does not effectively bind the pollutant and therefore does not alter its retardation. At high K_d^{om} or K_d^{cell} values, the partitioning of the carrier to the pollutant increases, but so does the partitioning of the carrier to the soil. The carrier therefore becomes less mobile than the pollutant itself and increases the retardation of the pollutant dramatically.

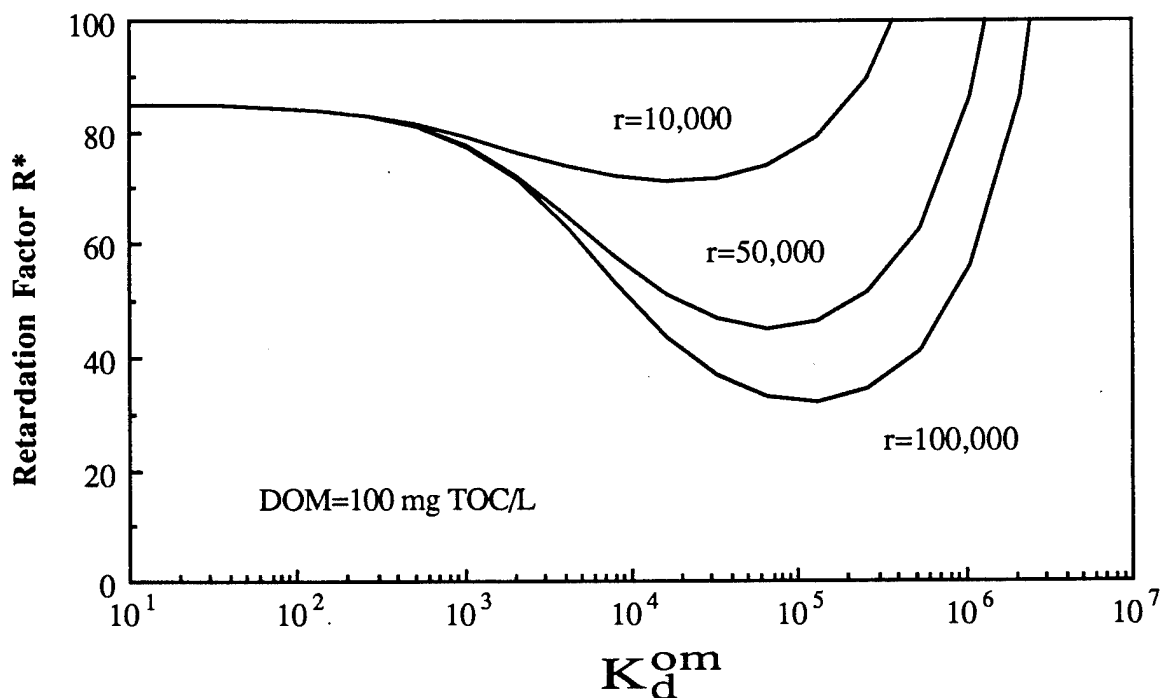


Figure 3. Effect of the partition coefficient between the pollutant and the carrier on the retardation factor of a hydrophobic pollutant as a function of the ratio of the carrier partition coefficient with the pollutant to the carrier partition coefficient with the stationary phase, $r = K_d^{om} / K_d^s$. [All calculations assume the sorbent and sorbate combination are comparable to phenanthrene and an aquifer sand, $K_d^s = 12.9$ and $\rho_b/n = 4.38$.]

If a particular value of the partition coefficient ratio (r) is assumed, then the influence of the carrier concentration may also be illustrated using the three-component model (see Figure 4). In general, increased carrier concentration results in increased pollutant mobility (lower R^*) as expected.

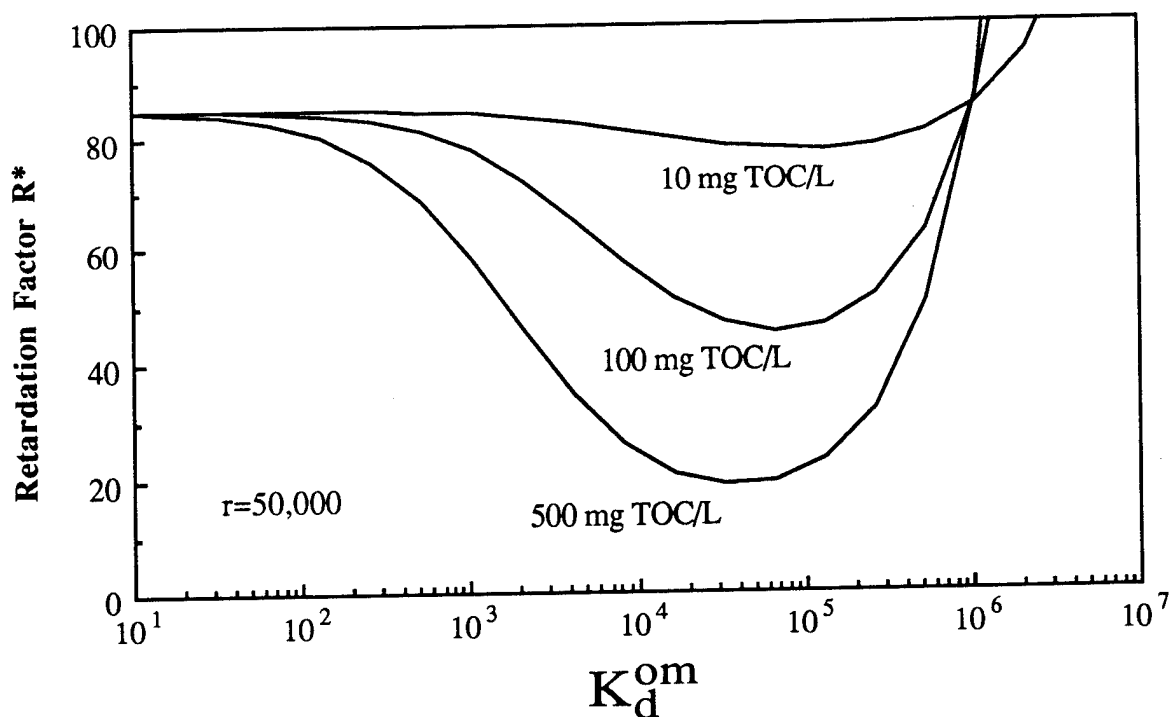


Figure 4. Effect of the partition coefficient between the pollutant and the carrier on the retardation factor of a hydrophobic pollutant as a function of carrier concentration. [All calculations assume the sorbent and sorbate combination are comparable to phenanthrene and an aquifer sand, $K_d^s = 12.9$ and $\rho_b/n = 4.38$.]

The above calculations illustrate that for a colloid or macromolecule to act as a carrier it must be mobile and able to bind the pollutant. These reactions act counter to one another since the more a carrier is able to bind organic pollutants by means of hydrophobic interaction, the more likely it is that the transport of the carrier will be retarded by hydrophobic interaction with the porous media. An effective carrier would likely have to contain a hydrophobic component capable of binding organic pollutants as well as a hydrophilic component to enable relatively unretarded transport through the soil matrix. Macromolecular materials, such as humic and fulvic materials and anionic detergents, and colloidal materials such as mineral precipitates and bacterial cells have been observed to have limited retardation relative to conservative tracers 10, 15, 43. Some potential carriers have been shown to have a greater affinity for sorbing hydrophobic pollutants than soil organic matter on an organic carbon basis 12, 44. The net result may be facilitated transport of some pollutants in the presence of these types of colloidal matter or dissolved macromolecules. Magee et al. 12, 41 showed a

reduction in the retardation factor of phenanthrene by a factor of 1.8 in soil column experiments in the presence of water soluble organic material extracted from soil. Other investigators found similar results for various pollutants and colloids 9, 10, 11, 45. The presence of an organic surfactant has also been found to reduce the partitioning of hydrophobic compounds to the stationary particulate phase in batch isotherm experiments 25, 46, 47, 48, 49, 50, and therefore batch sorption experiments may serve as a means for screening or selecting suitable carriers.

A quantitative evaluation of carrier mediated transport may have practical applications beyond the explanation of observed contaminant behavior. Since transport of hydrophobic pollutants can dictate the time required for pump-and-treat remediation technologies, methods that increase the flushing rate of pollutants could be implemented to reduce the operating time of a facility. Modifications could either include *in situ* stimulation and release of biogenic or inorganic "carriers" already present in the soil or could involve their addition to the infiltrating waters.

If the factors resulting in facilitated transport of pollutants become known, risk assessment at spill sites will become more accurate. If facilitated transport is not accounted for, high risk sites (i.e., those with conditions that result in high pollutant mobility) may be overlooked and contaminant transport underestimated.

2. Microbial Polymers as Carriers

Bacteria are naturally present in all soil and subsurface systems, at population densities between 10^2 - 10^9 /g of soil 51, 52. Bacterial populations in soil are reported to vary with clay content 51, pH and metal concentrations 52 and tend to decrease in numbers with increasing clay content (at $\text{pH} \leq 5$) and increase in numbers with increasing sand content (at pH 6 to 7). Surface soils near chemical spills can have elevated populations of over 10 billion bacteria per square centimeter of soil surface area 53. Many subsurface bacteria are capable of producing extracellular and cell-bound polymeric materials that are generally polysaccharides but can also include lipids, glycolipids, lipopeptides, and lipoproteins 54, 55 and desiccation 56.

Bacterial polymers may be either intracellular, cell-wall associated, or extracellular, each serving a particular function. The primary function of extracellular polysaccharides is thought to be as an adhesion mechanism, but extracellular polymers may also protect the cell against phagocytosis, amoebic attacks, and bacteriophages 53.

The polymer production rate for a given bacterial species may vary according to growth substrate and nutrient limitations. Sutherland ⁵⁷ found increased production of polysaccharides for *E. Coli*, *Enterobacter aerogenes*, and a *Pseudomonas* strain when growth was limited by nitrogen, phosphorus, sulphur, or potassium. The polymer structure, however, was independent of nutrient limitations or carbon source. Some bacterial isolates can convert up to 60% of the growth substrate to extracellular polymer.

Extracellular polymers have been detected in some ground- and surface waters ¹³ and, when attached to soil surfaces, have been shown to decrease the extent of adhesion due to hydrophobic interactions ⁵⁸. Many bacterial polymers can act as surfactants that reduce surface and interfacial tension. Singer ⁵⁵ reported that 62% of the isolates obtained from different sources produced surface active agents during growth on a glucose medium. The potential use of microbial biproducts for industrial uses has been recognized and presently many are commercially available under names such as surfactin (from a *Bacillus subtilis* strain) and emulsan (from an *Arthrobacter* strain, ⁵⁹). Considerable research has been carried out on the use of biosurfactants to aid in enhanced oil recovery by emulsifying and/or dispersing oil that is difficult to remove ^{60, 61}.

Since surface-active polymers have both a hydrophilic and a hydrophobic component, they may potentially act as a carrier for hydrophobic pollutants. Carbohydrate chains from bacterial polymers have been shown to trap and bind both small and large molecular weight substances ⁶². Indigenous soil organisms could potentially be stimulated *in situ* by adding nutrient solutions to induce production of polymers for the purpose of mobilizing hydrophobic pollutants at a contaminated site. Alternately, an above ground reactor could be built to harvest a desired polymer that could then, in turn, be added to the groundwater system. This would allow for addition of a selected carrier and eliminate potential clogging problems due to increased biological activity ⁶³. Addition of naturally occurring bacterial polymers may be more acceptable from both environmental and regulatory standpoints than adding exogenic or synthetic surfactants to a soil system.

The success of direct additions of extracellular polymers to soils would depend, in part, on the subsequent degradation of added polymers by indigenous soil bacteria. Several researchers have found that microorganisms usually do not degrade their own exopolysaccharide ^{64, 65}. However, there is also evidence that bacteria can degrade the exopolymers of other bacterial species ⁶⁶.

Some investigations have indicated that, although many exopolymers are readily biodegradable, the degradation rate is slow compared to simpler metabolites. As anticipated, the rate of polymer degradation varies with the type of microorganism that produced the polymer and the type of environment the polymer is degraded in. The polymers produced by activated sludge organisms were found to have a BOD₅ (in wastewater) to COD ratio of 0.10, implying a low level of biodegradability⁶⁷. Martin and Richards⁶⁸ studied the degradation (in soil) of the extracellular polysaccharides of *Chromobacterium violaceum* and two unidentified soil bacteria. The exopolymer of *C. violaceum* was rather resistant to degradation: after one week 10% of the polymer was mineralized to CO₂ and after eight weeks 53% of the polymer had been degraded. The extracellular polymers of the soil bacteria were degraded more readily: 50% after one week and 70% after four weeks. The exopolymers (from *Arthrobacter viscosus*, *Azotobacter indicus*, *Bacillus subtilis*, *C. violaceum*, and three *Pseudomonas* strains) were investigated by Martens and Frankenberger⁶⁹ and found to be degraded relatively quickly in soil. After one to two weeks, the monosaccharide concentration in the soil was not significantly different from the soil control, but only 60 to 75% of the carbon was recovered as CO₂ after eight weeks, indicating conversion of exopolymer to new biomass. Obayashi and Gaudi⁷⁰ studied the degradation (in wastewater) of the polysaccharides produced by *Arthrobacter viscosus*, *Azotobacter vinelandii*, *Xanthomonas campestris*, and *Zoogloea ramigera*. Unacclimated wastewater cultures were able to grow on each of these polysaccharides within 2 to 10 days. After enrichment, the wastewater was able to rapidly degrade the polysaccharides: 80 to 93% of the polymer COD was removed within 8 to 22 hours.

Degradation of polymer may also be influenced by the sorption of polymers to soil particles, other organic matter, or metals. There has been little research in this area. Recently, Francis et al.⁷¹ found that the tridentate citrate complexes with cadmium, copper, and lead were completely resistant to degradation, and that this resistance was caused by the chemical nature of the complex, not by the toxicity of the metal. It is possible that the biodegradability of extracellular polymers will be similarly altered if the polymers are complexed with various metals.

3. Mobile Cells as Carriers

Over the last decade, researchers have demonstrated the occurrence of significant bacterial populations in groundwater and terrestrial subsurface environments^{51, 52, 72, 73}. Many indigenous bacteria may be transported by groundwater under both saturated or unsaturated conditions and could potentially act as carriers of hydrophobic pollutants. For colloidal solids such as bacteria to increase the mobility of hydrophobic

pollutants, the pollutant must bind to the bacterial cells, and the microorganisms must be more mobile than the contaminant. The sorption of organic pollutants to bacteria and the mobility of bacterial cells in porous media are therefore relevant criteria for bacterially facilitated transport of hydrophobic pollutants in the environment.

Sorption of hydrophobic compounds to microorganisms has been examined by several investigators, and is generally assumed to be a passive process that can be treated in a manner analogous to sorptive partitioning 21, 22, 23. Sorption of organic pollutants to cells is reversible although the kinetics of desorption may differ from sorption 23. It cannot be assumed, however, that all microorganisms passively sorb hydrophobic compounds with the same affinity. Some components of a microbial community may more readily sorb hydrophobic contaminants than others.

Bacteria are colloidal in size. Models for filtration 18 suggest that colloidal particles with diameters $\approx 1 \mu\text{m}$ (the size of many soil bacteria) are of an optimal size for transport. Field studies have, indeed, demonstrated the mobility of bacteria in soil 74 and groundwater systems 15. There is, however, little information on the efficacy of soil bacteria as carriers of hydrophobic pollutants.

The populations of mobile bacteria in soils might be increased in at least two ways: (1) by *in situ* stimulation of indigenous organisms through introduction of nutrients or growth substrates that select for mobile populations or (2) by application to soils of strains or populations that are cultured in engineered reactor systems. Virtually no information exists on which components of microbial communities might be stimulated *in situ* to facilitate transport of nonionic pollutants in subsurface material. The successful addition of bacterial cultures to soils for purposes of remediation would require that the organisms that are introduced be able to survive in the subsurface environment. There is considerable public resistance to the release of genetically engineered cell strains and, therefore, this may not be a viable option. However, the selection and culture of naturally occurring soil bacteria that enhance hydrophobic pollutant transport appears to be feasible.

4. The Effect of a Carrier on Pollutant Mineralization

Before carriers are employed to mobilize pollutants, the secondary effects of adding a carrier to a subsurface environment must be considered. Secondary effects could include changes in soil porosity, changes in the indigenous microbiota, and altered pollutant degradation rates. Research on the effect of surfactant materials on the degradation of hydrophobic pollutants has been inconclusive. Results appear to

vary depending upon the surfactant, the pollutant and the environment. Aronstein et al.⁴⁶ observed an increase in the mineralization of phenanthrene and biphenyl in the presence of low concentrations of a nonionic surfactant. Higher concentrations completely inhibited mineralization. Laha and Luthy⁵⁰ also observed inhibition of the mineralization of phenanthrene in the presence of a nonionic surfactant above the critical micelle concentration (cmc) while lower levels did not seem to effect degradation. Falatko and Novak⁴⁵ found that the polymer produced from a culture grown on glucose media inhibited mineralization of gasoline while polymer produced from a culture grown in a gasoline media did not effect mineralization. In another study Guerin and Boyd⁷⁵ observed that one bacterial isolate was capable of desorbing and utilizing sorbed fractions of naphthalene while another isolate was limited to metabolism of the aqueous fraction of naphthalene. Generalizations for a whole mixed culture system based on tests with monocultures, therefore, may not be appropriate. In the subsurface, the microbial community may be able to mineralize both sorbed and aqueous fractions of pollutants. The influence of a carrier on the bioavailability of dissolved or sorbed pollutants is, therefore, an additional factor that must be considered when the introduction or stimulation of elevated carrier concentrations is planned for purposes of pollutant mobilization.

SECTION II

EXPERIMENTAL METHODS AND MATERIALS

To fulfill the objectives of this research, the following experimental approach was used:

1. Batch screening experiments were conducted to assess the ability of the following biological carriers to decrease the partitioning of phenanthrene onto a low-carbon aquifer sand:

a. extracellular polymers, both free (sometimes referred to as slime polymers) and cell-bound (referred to as capsular polymers), produced by several wild-type soil isolates and well characterized laboratory strains of bacteria, and

b. whole cell suspensions of wild-type soil isolates and well characterized laboratory strains of bacteria.

2. Selected polymers were characterized for molecular size and functional groups, and selected cell strains were characterized for their surface hydrophobicity.

3. Batch tests were carried out with selected cells and polymers to evaluate their sorption to the low-carbon sand medium and miscible displacement column tests were conducted to verify their mobility in a packed bed of the porous medium.

4. Column tests were conducted to verify that mobile polymers and cell strains could alter phenanthrene transport.

5. Experimental results from batch and column experiments were compared with model calculations using the three-component model of Magee et al. 41.

A. REAGENTS AND CHEMICALS

1. Sorbate

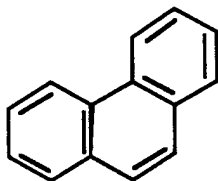
Phenanthrene was used in this research as a model nonionic organic compound. Phenanthrene is an EPA priority pollutant and is a common component of coal tar wastes and petroleum products such as kerosene ⁷⁶. Phenanthrene is a three ring polyaromatic hydrocarbon (PAH). In general, PAHs are relatively nonvolatile; they are strongly sorbed to soils through their hydrophobic interactions with soil

organic matter, and consequently are difficult to remove. The physical and chemical characteristics of phenanthrene are given in Table 1.

Table 1: Physical and Chemical Properties of Phenanthrene

Molecular weight (g)	178.24
Melting point (°C)	101
Boiling point (°C)	340
Density (g/cm ³)	0.98
Henry's Constant (dimensionless)	0.58277
Vapor Pressure (N/m ² @ STP)	0.0907
Log K _{ow}	4.4678
	4.573
Log K _{oc}	4.363
	3.7279
Solubility in water (mg/L)	1.293
Toxicity, mice (mg/kg) ^a :	
oral LD ₅₀	700
intravenous	56
skin TD _{LO}	71
skin TD	22,000

Structure:



Both stable (Sigma Chemical Co.; St. Louis, MO) and radiolabeled ¹⁴C-phenanthrene (Sigma Chemical Co.; specific activity = 13.1 mCi/mmol) were used in this research. Stock solutions of phenanthrene were tested for purity by high performance liquid chromatography and determined to be free of chemical and radiochemical impurities. Prior to batch and column experiments, phenanthrene crystals were placed in a saturator (see Figure 5) similar to that developed by Burris and MacIntyre⁸⁰ and dissolved in 18.9 MΩ/cm distilled-deionized water containing 5 mM CaSO₄ and 0.02% NaN₃. CaSO₄ was present as a background electrolyte and NaN₃ was added as a biological inhibitor. For mineralization experiments, the phenanthrene crystals were dissolved in a 50:50 mixture of methanol and distilled-deionized water.

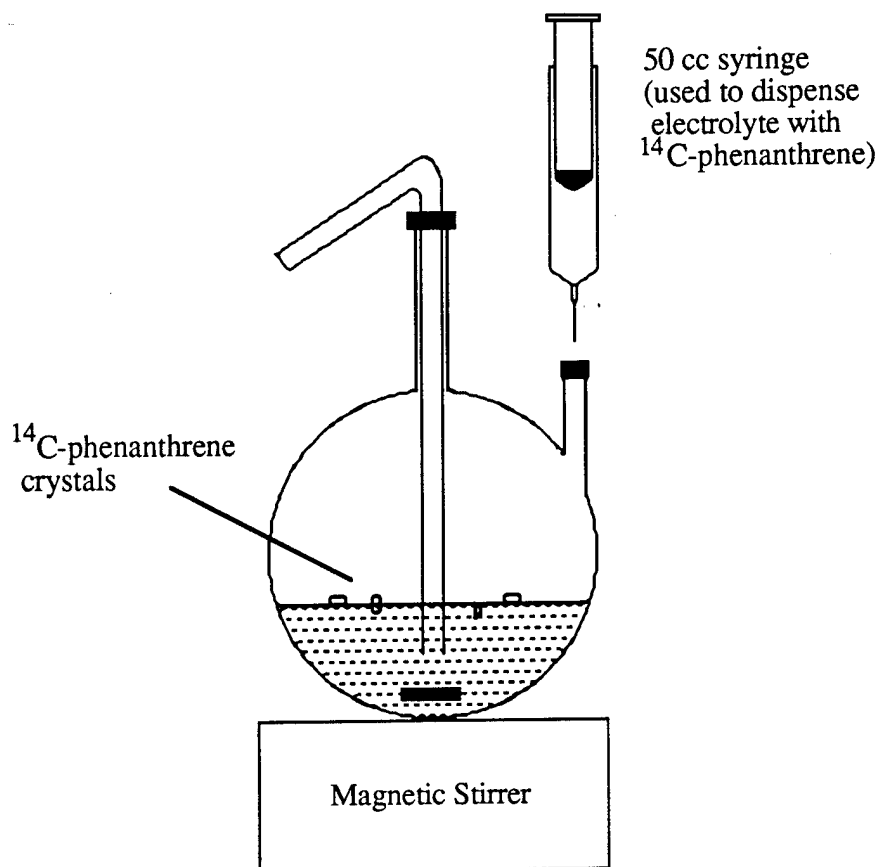


Figure 5. Saturator device used for preparation of phenanthrene solutions (after Burris and MacIntyre ⁸⁰).

2. Sorbent

The aquifer sand used in all experiments was obtained from a quarry in Newfield, New York. A particle size analysis of the sand was previously reported by Magee ⁸¹ and is shown in Table 2. Analysis of the sand was performed by the Cornell Nutrient Analysis Laboratory. Extractable cations and phosphate were determined by atomic absorption after extraction of the sand with a 10% sodium acetate in 3% acetic acid solution buffered to pH 4.8. Organic carbon was determined by the Walkley-Black method ⁸².

Table 2: Aquifer Sand Characteristics

Size Distribution

Silt and Clay, %	1.2
Sand, %	98.7
very fine (0.05 - 0.1 mm)	3.7
fine (0.1 - 0.25 mm)	47.2
medium (0.25 - 0.5 mm)	47.6
coarse (> 0.5 mm)	0.2

Elemental Analysis

mg/kg

Phosphate	1.8
Potassium	16
Magnesium	104.3
Calcium	17,453
Iron	25.5
Aluminum	19.3
Manganese	33.8
Copper	0.0
Zinc	0.06

pH in water 7.9

Organic Carbon, % 0.049

3. Bacterial Polymers

The bacterial isolates that served as sources of extracellular polymers were obtained primarily from a coal tar waste site and a laboratory culture collection (C. Thomas and W. Ghiorse; Section of Microbiology, Cornell University). Other cultures were obtained from the American Type Culture Collection (ATCC) and the Department of Energy Subsurface Microbial Culture Collection (D. Balkwill, Florida State University). One isolate from a soil root zone was also used⁸³. Sources of the isolates are indicated in Table 3. The isolates were stored in a 50/50 mixture of culture broth (described below) and glycerol at -70°C.

Table 3: Summary of Bacterial Isolates and their Sources

<u>Bacterium</u>	<u>Depth (m)</u>	<u>Gram reaction</u>	<u>Catalase</u>	<u>Oxidase</u>	<u>Source or Reference</u>
<i>Pseudomonas</i> A100	^a surface	-	+	+	83
9701A-2 ^b	1.8	-	+	+	84
9701A-4	1.8	-	+	+	84
9701M	1.8	-	+	+	84
9702A-2	4.0	-	-	+	84
9702M-4	4.0	-	-	+	84
9703A-1	11	+	+	-	84
9703M-5	11	+	+	+	84
9704M-1	12	-	+	-	84
9706M-2	4.8	+	+	-	84
9707M-3	7.0	+	+	-	84
9709A-3	9.4	+	+	-	84
9711A-2	5.8	+	+	-	84
9711A-4	5.8	+	+	-	84
9712M-3	8.1	+	+	-	84
9714A-4	9.1	+	+	-	84
B649 ^c	259	+	+	+	52
B693	259	+	+	+	52
<i>Pseudomonas cepacia</i> 249-100	N.A. ^d	-	+	+	85
<i>Pseudomonas fluorescens</i> ^e	N.A.	-	+	+	85

^a rhizosphere isolate from experimental soy bean plants cultured in Honeoye silt loam obtained from the Aurora research farm of Cornell University; Aurora, NY.

^b strains prefixed by "97" were isolated from an Electric Power Research Institute (EPRI) manufactured gas plant site in the midwestern US.

^c strains prefixed by "B6" were from the Department of Energy (DOE) field site at Savannah River Plant site P24; Aiken, SC. (DOE Subsurface Microbiology Collection at Florida State University)

^d N.A. = not applicable

^e ATCC 13524 (American Type Culture Collection; Rockville, MD.)

Distilled deionized water was used to prepare the growth medium (Table 4). The medium was autoclaved and adjusted to pH \approx 7. Approximately 300 mL of the broth was transferred into 1 L baffled flasks, inoculated with culture, and placed on a rotary shaker (at a constant temperature of 25°C) until stationary growth was attained (usually in 48 hrs).

Table 4: EPS Growth Medium

	<u>per L of media</u>
KNO ₃	0.7 g
MgSO ₄ 7H ₂ O	0.7 g
CaCl ₂ 2H ₂ O	0.14 g
Na ₂ HPO ₄ 7H ₂ O	1.61 g
KH ₂ PO ₄	0.78 g
Trace Metals	1 mL
3.6% Casamino Acid	10 mL
3.6% Yeast Extract	10 mL
0.4% FeNaEDTA	1 mL
Glucose	5 g
<u>Trace Metal Solution</u>	
Al ₂ (SO ₄) ₃ 24H ₂ O	35 mg
SnCl ₂ 2H ₂ O	13 mg
KI	13 mg
LiNO ₃	13 mg
MnSO ₄ H ₂ O	20 mg
H ₃ BO ₃	125 mg
ZnSO ₄ 7H ₂ O	25 mg
Co(NO ₃) ₂ 2H ₂ O	25 mg
CuCl ₂ 2H ₂ O	25 mg
NiCl ₂ 6H ₂ O	25 mg
Ba(OH) ₂ 8H ₂ O	13 mg
Na ₂ MoO ₄ 2H ₂ O	13 mg
KBr	25 mg

The procedure for extraction of extracellular polymers was adapted from techniques used by Corpe ⁸⁶ as described Kellems and Lion ⁸⁷. The culture broth was transferred into 250 mL centrifuge bottles and centrifuged for 25 minutes at 10,000 x g (5°C) to remove the cell mass. The supernatant was transferred into clean centrifuge bottles and mixed with equal volumes of 5°C acetone. Extracellular polymer was precipitated, forming a white floc, which was centrifuged at 3,000 x g for 15 minutes. The concentrated polymer solution was placed in 8000 molecular weight cutoff dialysis tubing (Spectra/Por[®], Spectrum Medical Industries Inc., Los Angeles, CA) and submerged in distilled and deionized water under constant mixing. Sufficient exchanges of water were made over a 48 hr period to remove excess solvents and low molecular weight impurities. The purified polymer was then frozen and freeze dried using a Flexi-Dry[®] Microprocessor Lyophilizer (FTS Systems Inc., Stone Ridge, NY).

Prior to use, the polymer was redissolved in the electrolyte solution (5 mM CaSO₄ + 0.02% NaN₃) at a concentration of 100 mg TOC/L as measured by an OI model 700 total organic carbon analyzer (College Station, TX). Polymer that was difficult to dissolve was sonicated and/or placed on a rotary shaker until dissolution was complete. Samples were then filtered through a 11 µm filter to eliminate flocculant polymer aggregates.

Cell-bound polymers were extracted according to a modified version of the procedure described by Brown and Lester ⁸⁸. Twenty-five mL of a 2% solution of Na₄EDTA was added to the cell mass after separation of the extracellular polymers as described above. After resuspending the cell mass, the solution was refrigerated for 30 minutes and centrifuged at 12,000 x g for 50 minutes at 5°C. The resulting supernatant contained the cell-bound polymers which were solubilized by EDTA treatment. The polymer solution was placed in 8000 MWCO dialysis tubing to remove low molecular weight impurities and then freeze dried.

Bacteria can produce polymers in the stationary as well as the exponential growth phase, depending on the isolate. Polymer production for selected isolates was measured as a function of cell concentration and the overall optical density of the broth culture. Cell mass was determined gravimetrically after washing of cell mass with electrolyte solution and drying at 105°C. Optical density was measured using a Beckman model 3600 spectrometer at a wavelength of 600 nm.

B. ISOTHERM EXPERIMENTS

Batch isotherms were used to determine the partitioning between phenanthrene and the low-carbon sand in both the presence and the absence of polymer, and the partitioning between polymer and the sand. All experiments were run in the dark at 25°C. The fluorescence quenching method described by Gauthier et al. ⁸⁹ was used to determine the partitioning between phenanthrene and the polymer.

1. Partitioning Between Phenanthrene and Sand

Flame-sealed borosilicate glass ampules (Wheaton; Millville, NJ) were used to provide an all-glass enclosure for the batch isotherm experiments since polynuclear aromatic hydrocarbons have been shown to significantly sorb to Teflon® seals ⁹⁰. The sand was placed on a tumbler prior to use to ensure sorbent homogeneity. Two types of isotherm experiments were conducted:

Variable amounts of sand: ampules received between 0 g and 4 g of sand and approximately 11 mL of either an electrolyte or an electrolyte/polymer solution. Sorbent mass and aqueous volume were measured gravimetrically.

¹⁴C-phenanthrene was removed from the stock saturator and placed in a 50 mL pear-shaped flask surrounded by crushed ice to reduce the volatilization of phenanthrene. One mL of ¹⁴C-phenanthrene was added to each ampule and the ampule was immediately flame sealed. ¹⁴C-Phenanthrene was also added directly to 10 mL of scintillation cocktail so that the sorption loss to experimental apparatus could be determined.

The ampules were placed in a constant temperature bath (25°C) and rotated about their vertical axis for 24 hours. A kinetic analysis indicated a 24 hour equilibration period was sufficient to obtain a close approach to equilibrium. Aqueous concentrations did not vary significantly for a period of 1-10 days ⁹¹.

After equilibration, the samples were centrifuged at 1000 x g for 60 minutes. One mL aliquots were removed and placed in 10 mL scintillation cocktail (Fisher Scintiverse E). The concentration of dissolved ¹⁴C-phenanthrene was measured as disintegrations per minute (DPM) by liquid scintillation using a Beckman LS 9800 Scintillation counter. Samples were counted for 30 minutes or until the variance was less than $\pm 2\%$.

Variable amounts of phenanthrene: A second type of isotherm was performed to see if a linear isotherm assumption was valid for aqueous phenanthrene concentrations approaching the solubility limit. A mixture of stable and radiolabeled phenanthrene was used to limit the quantity of radiotracer required. A saturated solution of stable phenanthrene, ranging from 0 - 8 mL, was pipetted into each ampule followed by 1 mL of radiolabeled phenanthrene. The remaining volume (up to 8 mL) was made up by addition of 5 mM CaSO_4 and 0.02% NaN_3 electrolyte solution. The ampules were immediately flame-sealed. Samples were then centrifuged at $1000 \times g$ for 45 minutes and counted for ^{14}C activity after a 24 hour equilibration period.

2. Partitioning Between Sand and Polymer

To determine the partition coefficient between the polymer and the sand, K_{dom}^s , a similar procedure was used as was followed in the phenanthrene/sand batch isotherms. Autoclaved, acid-washed, 10 mL screw-top test tubes were filled with 0, 2, and 4 g of sand respectively. Ten mL of polymer dissolved in electrolyte solution was added to the tubes and equilibrated for 24 hours at 25 °C on a rotating mixer. Samples were subsequently centrifuged at $2000 \times g$ for 15 minutes to remove suspended matter. The resulting supernatant was measured for total organic carbon. Dissolved polymer results were corrected for the amount of organic carbon released from the sand as measured from control samples without polymer.

3. Partitioning Between Phenanthrene and Polymer

The fluorescence quenching method described by Gauthier et al.⁸⁹ was used to determine the partition coefficient between phenanthrene and the polymer, K_d^{om} . Several investigators have previously used this method to measure the partition coefficient between PAHs and organic matter^{12, 29, 92}. The method is based on the principle that free soluble PAHs fluoresce while PAHs bound with dissolved organic matter (DOM), do not.

Fluorescence was measured using a SLM model 822/823 spectrofluorometer and excitation/emission wavelengths of 288 and 364 nm. A 2 mL sample of saturated phenanthrene solution was placed in a quartz fluorescence cuvette and allowed to equilibrate with the cuvette walls. The fluorescence was then measured. A series of 100 μL aliquots of polymer solution ($\approx 100 \text{ mg TOC/L}$) was added to the cuvette and allowed to equilibrate for 4 minutes before measuring the fluorescence. Polymer fluorescence was determined separately to correct the measured values for the fluorescence of the polymer alone.

C. CHARACTERIZATION OF POLYMERS

1. Infrared Spectra of Polymers

The infrared (IR) spectra of a polymer sample was measured using KBr pellets and a Perkin-Elmer Model 683 Infrared Spectrophotometer. Polymer samples were mixed with IR grade KBr (Sigma Chemicals) at a ratio of roughly 1:400 and freeze dried to remove any excess moisture. Pellets were pressed in a KBr dye (Perkin-Elmer) under 20,000 lbs pressure using a Carver laboratory press.

2. Size Exclusion Chromatography

High Performance Liquid Chromatography (HPLC) was used to determine the approximate molecular weight of the polymers. Analyses were performed using a Hewlett-Packard 1090 HPLC. A 300 x 7.5 mm BioSep-SEC™-S4000 column (Phenomenex, Torrance, CA) packed with 5 μ m bonded silica with 500 Å pore size was used with a mobile phase of 50 mM phosphate buffer at pH of 6.0. Samples were prepared by dissolving 50 mg of standard into 1.5 mL of distilled deionized water and filtering through a 0.2 μ m filter. Detection was provided by a diode array detector at wavelengths of 195 nm, 254 nm, and 275 nm with sample injection volumes of 100 μ L. A standard curve was prepared by using molecular weight standards ranging from 66,000 daltons (albumin) to 2,000,000 daltons (blue dextran).

D. COLUMN EXPERIMENTS

Miscible displacement experiments were performed to determine the retardation of phenanthrene in porous media and to quantify the effect of polymer on the transport of phenanthrene.

Two syringe pumps (Pharmacia LKB P-500) were used: one to deliver the phenanthrene pulse and the second to deliver the mobile phase. To prevent losses by volatilization, the phenanthrene solution for the input feed was placed in a serum vial sealed with a Teflon® septum. This vial was also connected via a glass tube to the headspace of a larger vial containing a saturated phenanthrene solution in order to decrease the extent of the vacuum formed as the phenanthrene input pulse was fed into the column (see Figure 6).

The two syringe pumps were connected to a six-port switching valve (Rheodyne 7000) to allow for an immediate switchover from or to the pulse of phenanthrene while keeping both lines flowing. The experimental setup is shown in Figure 6.

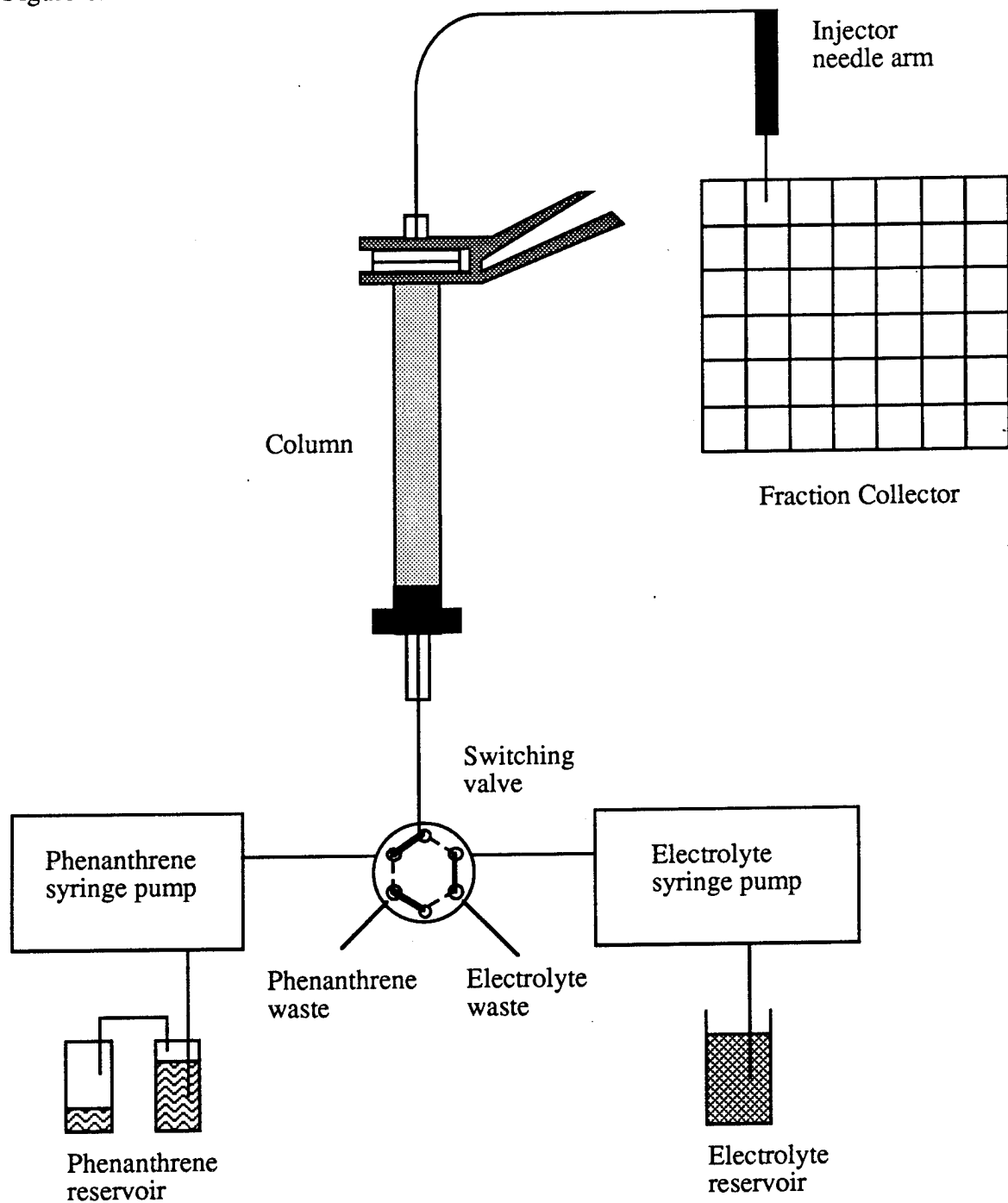


Figure 6. Schematic of assembly used for miscible displacement experiments.

The eluent from the syringe pump used to deliver phenanthrene to the column came into contact with several fluoroplastic components (Kel-F®, Tefzel®, and Teflon®) of the pump onto which the phenanthrene may sorb. These lines were presaturated with phenanthrene for approximately 12 hours prior to the initiation of a column experiment by circulating the pump effluent back to the reservoir. Stainless steel tubing was used wherever possible to limit the sorption of phenanthrene onto the experimental apparatus.

An "all-glass" column assembly comparable to that described by Lion et al.⁹⁰ was employed to minimize the use of fluoroplastics. It consisted of a millipore fritted end piece blown to an Ace threaded end. A detailed schematic of the column is shown in Figure 7.

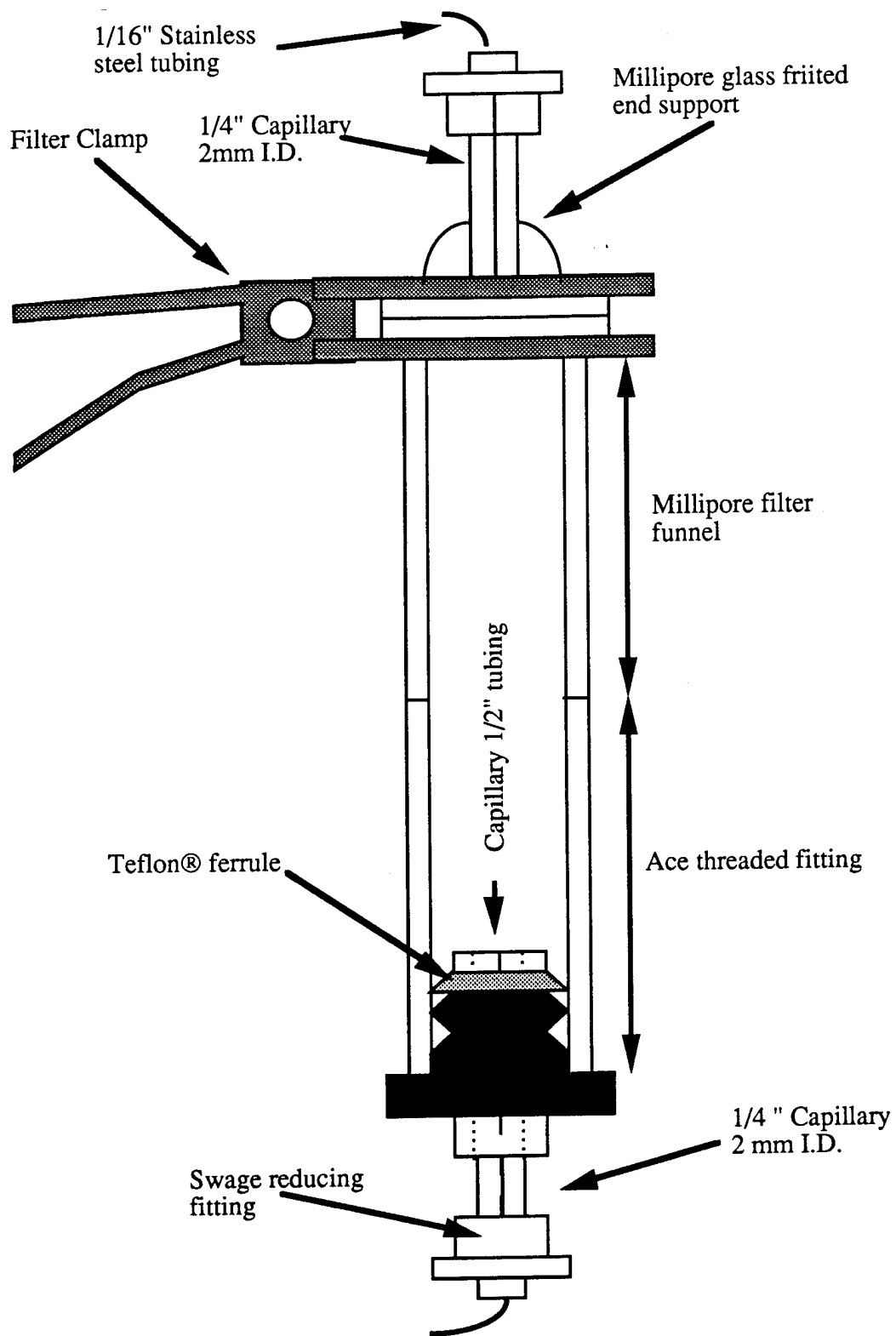


Figure 7. Custom column apparatus designed by Lion et al. 90 to minimize hydrophobic fittings.

The column was prepared for each experiment using the following steps:

1. The column was washed in a nitric acid bath and rinsed with deionized water.
2. A 2.7 μm glass-fiber filter (precleaned by combustion in a muffle furnace) was placed over the tubing as a bed support to prevent sand from entering the tubing.
3. The empty dry weight of the column was measured.
4. Sand was placed in a tumbler to achieve homogeneity.
5. Small amounts of sand were added to the column while gently tapping the sides.
6. The fritted end piece was clamped onto the filled column.
7. The filled column was weighed to measure dry weight of the low-carbon sand.
8. The column was saturated with at least 50 pore volumes of electrolyte solution.
9. The saturated column was weighed.

The total pore volume of the column was determined by the weight difference of the saturated and dry column after correction for the dead volume in the tubing and end pieces.

A chloride pulse was performed on each column to confirm gravimetric estimates of the pore volume. Chloride breakthrough data was also fit to a least squares solution to the advection dispersion equation ⁹³ to determine the pore velocity and the chloride dispersion coefficient.

A 6 hour pulse of phenanthrene was added to the column at a bulk flow rate of 5 mL/hr (pore velocity approximately 7.2 cm/hr). The column effluent was directed to a fraction collector (Gilson model 222) where 1.5 mL samples were delivered into aluminum foil covered vials containing 10 mL of scintillation cocktail (Fisher Scintiverse E). To minimize loss by volatilization of phenanthrene, the delivery

position of the fraction collector was adjusted so that the column effluent was discharged below the surface of the scintillation cocktail. The activity of the cocktail solution was measured for 30 minutes or until the counts were within $\pm 2\%$, which ever came first, using a Beckman LS 9800 Scintillation counter.

E. PHENANTHRENE MINERALIZATION

Mineralization tests (@ 25°C) were performed on a selected polymer to test its effect on the degradation of phenanthrene. Autoclaved 155 mL serum bottles were used in all experiments. The bottle assembly contained a glass Pasteur pipet that was blown to form a 500 μL vessel and filled with 0.5 M NaOH to trap the $^{14}\text{CO}_2$ released upon mineralization of ^{14}C -phenanthrene. A mixed culture inoculum was obtained by enriching indigenous bacteria from one gram of soil (heavily contaminated with coal tar waste) in a mineral salt medium (Table 5). Sand and growth medium were added at a ratio of 1:5 and autoclaved. ^{14}C -Phenanthrene dissolved in a 50:50 mixture of methanol and deionized water was added aseptically to the serum bottle and immediately crimp capped and allowed to equilibrate with the sand for 24 hours before inoculating with the enriched mixed culture. Solvophobic effects due to the presence of methanol were considered insignificant because of the small final concentration of methanol. The vial septa were presaturated with stable phenanthrene vapor to minimize sorption losses. Control experiments were performed by adding sodium azide to attain a concentration of 0.02%. During each mineralization experiment, the NaOH solution was removed from bottles with a 500 μL syringe and immediately injected into 8 mL of scintillation cocktail containing 100 μL glacial acetic acid (to eliminate chemoluminescence), after which the ^{14}C activity was determined using a Beckmann LS 9800 Scintillation counter. Fresh NaOH was then injected through the septa into the bottle. This process was continued until no significant ^{14}C accumulation was detectable in the NaOH solution. Sample bottles were then allowed to cool to 4°C to reduce the amount of phenanthrene vapor present. The solution was then removed and placed in scintillation cocktail to give an indication of the total mass of ^{14}C recovered.

Table 5: Growth Medium (mg/L) for Mineralization Studies

KH_2PO_4	900
K_2HPO_4	100
NH_4NO_3	200
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	100
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	100
FeCl_3	10

F. EXPERIMENTS WITH BACTERIAL ISOLATES

The bacterial isolates that were screened (using suspensions of whole cells) for their influence on the sorption of phenanthrene to the low-carbon sand sorbent include all those previously listed in Table 3. Additional isolates that were tested in the experiments with whole cell suspensions are listed in Table 6. Isolates B121, and B550, were obtained from Dr. P. Baveye (Department of Soil, Crop, and Atmospheric Science, Cornell University). The *Bacillus subtilis* strain, isolates N1, and Nd9, and isolates *Methylamonas albus* BG8 (ATCC) [a Type I methanotroph]; *Methylosinus trichosporium* OB3b (ATCC) [a Type II methanotroph]; and *Methylocystus parvus* OBBP (LB) [a Type II methanotroph] were obtained from Dr. W.C. Ghiorse (Section of Microbiology, Cornell, University). [Note that, until recently, Type I and II methanotrophic bacteria were distinguished, in part, by their ability to produce the soluble form of the enzyme methane monooxygenase (MMO). MMO in Type I methanotrophs was thought to be membrane bound. However Koh et al. [Koh, 1993 #511] have recently demonstrated production of soluble MMO by a Type I methanotroph, somewhat blurring the distinction between these two groups of bacteria.]

A peptone-tryptone-yeast extract-glucose (PTYG) medium consisting of (L⁻¹) 5 grams peptone, 5 grams tryptone, 10 grams yeast extract, 10 grams glucose, 0.6 grams K₂HPO₄, 0.07 grams CaCl₂·2H₂O, with pH adjusted to 7 was used for initial cultures of nonmethanotrophic cell strains. A single colony of an isolate from a fresh full-strength or 5% PTYG agar slant or plate was used to inoculate a small volume of either full strength or 5% PTYG broth which was then incubated at 25 °C on a rotary shaker at 180 rpm until the stationary growth phase. A 10 mL volume of this culture was then used to inoculate 250 mL of PTYG broth which was incubated as described above. This volume of cells was then harvested and used in experiments. The cells were centrifuged at 8,000 to 10,000 x g for 20 to 30 minutes, and washed twice in 100 mL of sterile 5 mM solution of CaSO₄ with or without 0.02% NaN₃. After the second wash, the cells were resuspended in 100 mL of sterile 5 mM CaSO₄ and starved for 24 to 48 hours on a rotary shaker at 180 rpm and 25 °C. Starved cells were harvested by centrifugation, and resuspended in 5 mM CaSO₄ with or without 0.02% NaN₃. Cell densities were determined using a Coulter Counter, by measuring cell dry weights, or by total organic carbon (TOC) analysis using an O.I. Corporation Model 700 TOC Analyzer.

Table 6. Additional Bacterial Strains Used in Experiments with Whole Cells.

Bacterial strain	Depth (m)	Gram reaction	Catalase	Oxidase	Source or reference
<i>Arthrobacter globiformis</i> 8010	NA ^a	+	+	ND ^b	ATCC ^c
<i>Acinetobacter calcoaceticus</i> 31012	NA	-	+	-	ATCC
<i>Bacillus subtilis</i>	NA	+	+	+	W. Ghiorse
<i>Methylosinus trichosporium</i> OB3b	NA	-	+	+	W. Ghiorse
<i>Methylocystis parvis</i> OBBP	NA	-	+	+	W. Ghiorse
<i>Methylomonas albus</i> BG8	NA	-	+	+	W. Ghiorse
<i>Zoogloea</i> WNJ8	NA	-	+	+	85
B121 ^d	ND	-	+	+	52
B550	ND	+	+	+	52
N1 ^e	surface	-	+	-	84
Nd9 ^f	surface	-	+	+	W. Ghiorse

^aNA = not applicable.

^bND = no data.

^cAmerican Type Culture Collection, Rockville, MD.

^dStrains prefixed by "B" were from the Department of Energy (DOE) field site at Savannah River Plant site P24, Aiken, SC. (DOE subsurface Microbiology Collection at Florida State University)

^eA naphthalene metabolizing strain isolated from a surface soil environment at a forested site contaminated with coal-tar residues.

^fA naphthalene metabolizing strain isolated from a DOE field site at Hanford Washington.

The strains of methanotrophic bacteria were transferred to plates of minimal nitrate salts medium (NSM) described in Graham et al. ⁹⁴, ie.: NaNO₃, 1.0 mM; K₂SO₄, 1.0 mM; MgSO₄-7H₂O, 0.15 mM; CaCl₂-6H₂O, 47.6 μM; KH₂PO₄, 3.9 mM; Na₂HPO₄, 6.0 mM; FeSO₄-5H₂O, 40 μM; Na₄EDTA, 50 μM; ZnSO₄-7H₂O, 2 μM; MnSO₄-H₂O, 1.6 μM; H₃BO₃, 6.0 μM; NaMoO₄-2H₂O, 0.4 μM; CoCl₂-6H₂O, 0.4 μM; KI, 1.0 μM; CuSO₄-5H₂O, 1.0 μM. The phosphate salts were autoclaved separately and added after cooling to 50°C. 1.5 % Bacto Agar from Difco was added when plates were poured. Inoculated plates were put into a vented Gas-Pak[®], and exposed to a 1 to 1 mixture of methane and oxygen with a trace of carbon dioxide. The sealed Gas-Pak[®] was placed in a 30°C incubator.

Fresh plates were used to inoculate 25 mL of NSM in sterile serum bottles which were crimp-sealed with sterile rubber septa. The air in the bottles was replaced, under aseptic conditions, with the same mix of methane, oxygen, and carbon dioxide as used in the Gas-Pak. The inoculated bottles were placed in a rotary shaker at 120 rpm in a 25 °C incubator. Growth in these bottles was used as inocula for production of 500 mL batches of cells in 1 liter screw-cap culture bottles. The cultures of methanotrophic cells were used for obtaining extracellular polymeric material and washed cells for batch isotherm experiments with phenanthrene. The bottles were fitted with a custom seal so that they could be supplied with a methane, oxygen, carbon dioxide mix, the methane being the sole carbon and energy source for the obligate methane oxidizers. Cells of the methanotrophs were harvested by centrifugation as described above. The supernatant was saved, and was dialyzed directly (without precipitation of polymers by acetone) in Spectra/Por Membrane MWCO: 6-8,000 tubing. The dialyzate was freeze-dried, and recovered polymer stored at 4°C.

1. Culture Characterization

Microscopic examination: Selected subsurface bacteria were examined by light microscopy using standard techniques and staining reagents (from Fisher Scientific) for motility, and gram reaction. A Zeiss D-7082 model microscope was used. Cell dimensions were also determined by light microscopy.

Taxonomic characterization: Two isolates, 9702M-4 and 9702A-2, were grown on 5% PTYG agar plates. API, rapid NFT strips, designed to identify nonfermenting gram (-) bacteria, were used for their characterization.

Electron microscopy: Isolates 9702A-2 and 9702M-4, were grown in 5% PTYG to early stationary phase. Samples were then prepared for EM examination by negatively staining with 1% potassium phospho-tungsten and 50 mM bacitracin. The isolates were examined using a Phillips 201 TEM 80 KV instrument.

Specific growth rate determination: Single colonies of isolates 9702M-4 and 9702A-2 were taken off fresh 5% PTYG plates and used to inoculate 50 mL of 5% PTYG broth. The broth cultures were incubated on a rotary shaker at 30°C and 200 RPM over night. These two starter cultures were in exponential growth phase when used to initiate the specific growth rate experiment. Triplicate side arm flasks containing 50 mL of 5% PTYG broth were inoculated with a quantity of starter culture that brought the initial OD₆₀₀ to 0.09 to 0.1. These flasks were incubated in the same manner as the starter cultures. A Spectronic 20 was used to take optical density

(OD₆₀₀) readings at approximately two hour intervals. The experiment was terminated when the last two optical density readings were the same, and growth had ceased. Specific growth rates were determined using the equation $\mu = \Delta \ln OD_{600} / \Delta t$, where t = minutes, and data from the linear part of the time vs. $\ln OD_{600}$ plot was used. The generation times (G) were calculated using $G = \ln 2 / \mu$. Results were expressed as the means of replicate determinations.

Test for Cell Surface Hydrophobicity: The hydrophobicity of selected bacterial isolates was quantified by the microbial adhesion to hydrocarbons (MATH) test as described by Rosenberg et al. 95, and Rosenberg 60. The following organisms were tested: *A. calcoaceticus* (ATCC 31012), and soil isolates 9701A-2, 9702A-2, 9703A-1, 9709A-3, 9711A-2, 9712M-3, A100, Nd9, and N1. All glassware used in this test was acid washed, and autoclaved. Bacterial cells were grown and harvested as described above (report section F) with the following changes. The cells were washed 2x in 0.15 M phosphate buffer (PB) at pH 7, resuspended in PB, and adjusted to a cell density of OD₆₆₀ 1.5. A volume of 2.4 mL of cell suspension was aseptically pipetted into round-bottomed screw-cap culture tubes. In duplicate, 100, 200, and 400 μ L of hexadecane (Sigma Chemical Co.) were pipetted into the cell suspensions. The tubes were then incubated in a water bath at 30°C for 15 minutes. After vortexing the tubes for 120 seconds, they were again placed in the 30°C water bath for 15 minutes to allow the two phases to separate. The final optical density (OD₆₆₀) of the cell suspensions was determined using a Bausch and Lomb Spectronic 20.

Mineralization of phenanthrene: Isolates 9702M-4 and 9702A-2 were tested for their ability to metabolize phenanthrene. Cells were grown on 5% PTYG agar plates. Fresh colonies were transferred to 3 mL of a mineral salts medium (Table 5) in a sterile 10 mL serum vial into which was placed a small marble for agitation, and a glass reservoir for 500 μ L of a 0.5 M solution of NaOH to trap CO₂. 100 μ L of a ¹⁴C-phenanthrene spiked saturated aqueous solution of phenanthrene was added to the vials, crimp-sealed with a teflon septum, and incubated at 30°C on an orbital shaker for 36 hours. The NaOH was then removed with a syringe and transferred to scintillation cocktail and radioactivity was read in a scintillation counter. Two controls were used: 1. no phenanthrene added; 2. ¹⁴C-phenanthrene added to a suspension of a known phenanthrene degrader.

Test for Soluble Methane Monooxygenase Activity of OB3b and its Ability to Oxidize Phenanthrene: The methanotrophic isolate OB3b was grown in the low nitrate mineral salts medium (NSM), as described above, but without the addition of Cu. OB3b synthesizes soluble methane monooxygenase (MMO) and not the

particulate form of MMO under conditions of low [Cu]. A fresh culture of OB3b growing on NSM plates (which included Cu in the trace element solution) was used to inoculate 30 mL of -Cu NSM in a serum bottle. A mixture of methane, oxygen, and carbon dioxide filled the head-space. After sufficient growth was visible, one mL of this suspension was used to inoculate a fresh serum bottle of -Cu NSM. The methane, oxygen, and carbon dioxide mixture was replenished every other day until a cell density approximating an A_{600} of 1.0 was obtained. Following the method of Brusseau et al. 96, the suspension of OB3b was diluted with fresh -Cu NSM to an A_{600} of 0.2. Crystals of naphthalene and phenanthrene were placed in sterile Hungate tubes, each in triplicate. Three mL of the diluted cell suspension were pipetted into the tubes which were sealed with septa and crimp-caps and incubated at 30 °C on a shaker at 200 rpm for one hour. The following controls were used: (1) cells without PAH, and (2) naphthalene and phenanthrene in -Cu NSM. The reaction mixtures were transferred to screw-cap tubes, 100 μ L of 4.21 mM of o-dianisidine was added; the tubes were capped, mixed by vortex, and read immediately in a Spectronic 20 at 525 nm.

2. Whole Cell Phenanthrene Sorption Isotherms

In all batch isotherm experiments funnel-top borosilicate ampoules (Wheaton) were used to contain a maximum volume of 12 mL, 11 mL of a 5 mM CaSO_4 , 0.02% NaN_3 solution, with or without a suspension of bacterial cells, and 1 mL $1.5 \pm 0.1 \mu\text{M}$ of ^{14}C -phenanthrene. The ampoules were flame sealed immediately after the addition of the ^{14}C phenanthrene. Sealed ampoules were equilibrated at a constant temperature of 25 °C on a slowly rotating tumbler. After equilibration, cells were removed from suspension by centrifugation ($\sim 330 \times g$ for 30 minutes), the ampoules were opened, one mL of the electrolyte was transferred to 10 mL of scintillation fluid (ScintiVerse, Fisher Scientific), and the ^{14}C activity for each sample was determined on a Beckman LS 9800 Scintillation counter. ^{14}C activity was corrected for background and quench. Cell densities of the dilutions pipetted into the ampoules were measured before and after the period of equilibration. In all cases there was no indication of changes in cell mass. NaN_3 , a respiratory inhibitor, was used to prevent active uptake and metabolism of phenanthrene. The constancy of cell mass in experiments was taken as evidence that azide also inhibited replication.

In experiments with sand present, the sand was separated from suspensions with phenanthrene (with or without added cells) by sedimentation for 24 hours. The aquifer sand used in experiments is characterized in Table 2. Sorption of phenanthrene to the aquifer sand in the presence and absence of suspended cells was evaluated using the following linearized form of the mass-balance equation (41):

$$C_0V_0/C_sV_s = 1 + K_d(M/V_s) \quad (11)$$

where: C_0 is initial concentration, in DPM, of ^{14}C -phenanthrene,
 V_0 is volume of solution,
 C_s is concentration of sorbate in equilibrium with sorbent,
 V_s is volume of solution containing the sorbent,
 K_d is the linear distribution coefficient; and
 M is mass of sorbent.

Plotting M/V_s on the x axis, and C_0V_0/C_sV_s on the y axis yields a linear slope that is equal to the distribution coefficient (K_d) for the solute with the sorbent.

Soil isolate 9710M-3 was used to examine the kinetics for sorption of phenanthrene (sorbate) to bacterial cells (sorbent). Duplicate samples were removed at 0, 4, 8, 12, 24, and 48 h, and the ^{14}C activity was determined. Twenty-four hours proved sufficient for reaching equilibrium. Isotherm experiments with 3 concentrations of cells (9710M-3) with and without NaN_3 indicated that sodium azide did not affect the value of distribution coefficient of phenanthrene with the cells. Thus, NaN_3 was used in all subsequent batch isotherm experiments. For determining the effect of cells on the K_d value of phenanthrene with the aquifer sand, three quantities of the sand (generally 1, 2, and 3 grams), and three concentrations of cells, were used with two or three replicates of each treatment.

3. Bacterial Cell Mobility in Porous Media

The isolates were grown and harvested as described above (report section F). Plate counts and total organic carbon (TOC) content of the cell suspensions were determined. Dimensions of the starved cells were determined using a Zeiss Universal transmitted-light microscope. An autoclavable syringe transfer pipet was filled and dry packed with aquifer sand after Whatman #40 filter paper was placed over the outflow orifice. Another piece of filter paper was placed over the exposed sand, and a #2 rubber stopper with a 6 cm length of 0.064 cm (outer diameter) stainless steel syringe tubing through it was used to cap the sand column. The column was mounted in a vertical position. Bulk density of the aquifer sand was determined gravimetrically. After the column was saturated with a solution of 5 mM CaSO_4 , it and the tubing connecting it with the reservoir and fraction collector were autoclaved for 2 hours. Sterility of the system was checked before initiating an experiment by pumping sterile 5 mM CaSO_4 through the tubing and column and plating the effluent on 5% PTYG agar medium. These plates were incubated at 2 °C; absence of growth after two days was taken as grounds to initiate a mobility experiment. Using a peristaltic pump at a flow

rate of 6 to 12 mL/hour, a 5mM CaSO₄ solution was pumped upward through the column. Autoclavable nalgene tubing was used to deliver the effluent to a fraction collector (Gilson Model 222). The length of the packed column was 6.5 cm, and the bulk density of the packed sand was ~1.60 g cm⁻³.

The column apparatus was set up under a laminar flow transfer hood to decrease the probability of contamination. A chloride break through curve (BTC) was performed separately before initiating the BTC for the cells. A sterile 0.085 M solution of NaCl in 5 mM CaSO₄ was applied to the column for two h followed by several pore volumes of a sterile 5 mM CaSO₄ solution. Like the chloride BTC, the cell suspension was injected into the column as a 2 or 6 hour pulse; the pulse was followed by a continuous injection of sterile 5 mM CaSO₄.

Chloride concentration was determined using standard argentometric methods. Cell density was determined using plate counts and TOC determinations. Based on the Cl⁻ BTC and using CXTFIT, a model developed by Parker and van Genuchten⁹³, the dispersion coefficient, and pore water velocity were estimated. The retardation coefficient (R) for the isolates was estimated using the first temporal moment of the BTC:

$$R = [(\sum(C/C_0)\theta d\theta)/(\sum(C/C_0)d\theta)] - \theta_p/2 \quad (12)$$

where: C is the effluent concentration of cells,
C₀ is the influent concentration of cells,
 θ is time expressed as pore volumes, and
 θ_p is the duration in pore volumes of the pulse of cells.

The distribution coefficient for the bacterial cells with the sand (K_{cell}^s) was calculated from R using the equation:

$$R = 1 + K_{cell}^s \rho/n \quad (13)$$

where: ρ is bulk density of the sand,
K_{cell}^s is the distribution coefficient, and
n is porosity.

4. Column Studies with Phenanthrene in the Presence and Absence of Cells

Glass column. An all-glass column assembly, as described by Lion et al.⁹⁰ was acid-washed; and the dead volume was determined gravimetrically. Whatman GF/D glass microfibre filter paper (2.7 μ m) was combusted at 550 °C to remove carbonaceous material, and placed at the inlet end of the column before it was

dry packed, under conditions of constant tamping, with the aquifer sand. The length of the packed column was ~ 5 cm, and the bulk density was determined by weight to be between 1.5 and 1.6 g cm⁻³. The column was connected to a continuous flow syringe pump (Pharmacia LKB-Pump P-500), and a fraction collector (Gilson Model 222). The column was housed in a constant temperature chamber that was kept at 25 °C. Before initiating a BTC, the column was saturated with a solution of 5 mM CaSO₄ and 0.02% NaN₃. A chloride BTC preceded the phenanthrene BTC.

For the phenanthrene BTC, 0.42 μM ¹⁴C-phenanthrene (~12,000 DPM mL⁻¹) was applied to the column for 6 h, and followed by 5 mM CaSO₄, 0.02% NaN₃. When a bacterial culture was incorporated into the miscible displacement experiments, washed and starved cells of known cell concentration were mixed with the ¹⁴C-phenanthrene used in the pulse which was then chased with a suspension of cells of the same cell concentration. The flow rate of the syringe pump was 5 mL/hour. The concentration of the cell suspension was ~65 μg TOC mL⁻¹ (~5 x 10⁷ cells mL⁻¹) in all experiments. One mL fractions of eluate were collected directly into scintillation cocktail. Concentration of the radiolabel was determined using a Beckman LS 9800 liquid scintillation counter. CXTFIT⁹³ was used to determine the pore water velocity, and the dispersion coefficient based on data from the chloride BTC. Retardation of phenanthrene was estimated using Equation (12) for the first temporal moment.

Stainless steel column. A stainless steel chromatography column, 12.5 cm by 1 cm I.D., with all steel fittings and 2 micrometer stainless steel frits (Alltech), was used in place of the all glass column for several column experiments. It was less susceptible to leaking, an important factor in recovering radio-labeled chemicals. This steel column was used in a desorption experiment, and in phenanthrene BTCs with methanotrophs. In the desorption experiment, cells were not included in the pulse of labeled phenanthrene. Thus, a pulse of phenanthrene in the electrolyte solution was followed by a suspension of cells, as in the other packed column experiments, until a 100% of labeled phenanthrene was recovered.

SECTION III

RESULTS

A. ISOTHERM DATA ANALYSIS

The mass balance equation of a pollutant in a saturated porous media can be written as follows:

$$\left(\begin{array}{c} \text{Total mass of} \\ \text{compound in} \\ \text{control volume} \end{array} \right) = \left(\begin{array}{c} \text{Mass of compound} \\ \text{in the aqueous phase} \\ \text{in control volume} \end{array} \right) + \left(\begin{array}{c} \text{Mass of compound} \\ \text{bound to solids in} \\ \text{control volume} \end{array} \right)$$

$$C_o V_o = C_s V_s + X \quad (14)$$

If sorption is assumed to obey a linear isotherm, then

$$X = K_d C_s M \quad (15)$$

where: C_o is the aqueous concentration of compound in control without sorbent,

V_o is the total liquid volume of control,

C_s is the aqueous concentration of compound in sample with sorbent,

V_s is the liquid volume in sample,

K_d is the partition coefficient, and

M is the mass of sorbent in sample.

To obtain a linear relationship between the measured parameters with a slope equal to K_d , the above equation can be rearranged in two separate ways:

$$\frac{C_o V_o - C_s V_s}{M} = C_s K_d \quad (16)$$

$$\frac{C_o V_o}{C_s V_s} = 1 + \frac{M}{V_s} K_d \quad (17)$$

Equation (16) is commonly found in the literature. However, for typical isotherm experiments, the aqueous concentration C_s is the measured parameter with the highest margin of error since both volume and mass may be measured gravimetrically. A linear regression using Equation (16) would violate two assumptions inherent in the regression of the data: 1) the assumption that the x variable (C_s) is known without error and 2) the assumption that the y variable is measured independently of x (C_s appears on

both sides of the equation). In Equation (16), although there is some interdependence of x and y (from V_s), it is minimal. Equation (17) would therefore be anticipated to be more appropriate in estimating the actual distribution coefficient from the slope of the line. The value of K_d can vary greatly depending on which equation is used. For example, using a single data set, estimated K_d values using Equations (16) and (17) were 17.8 mL/g ($r^2=0.983$) and 20.6 mL/g ($r^2=0.993$), respectively. Unless otherwise indicated, all calculations were performed using Equation (17).

B. SORPTION OF PHENANTHRENE

1. In the Absence of Polymer

The average value obtained for the distribution coefficient for phenanthrene with the sand, K_d , was 20.5 ± 3.0 mL/g ($n=43$) which, on an organic carbon basis, gives $K_{oc} = 42,200 \pm 8000$ mL/g. K_{oc} values for phenanthrene measured by other researchers include 22,900 mL/g for a river sediment ³ and 9800 mL/g on humic acid ⁹². Karickhoff et al. ³ and Schwarzenbach and Westall ⁹⁷ found that correlations between f_{oc} and K_d break down at $f_{oc} = 0.001$. Mineral surfaces and organic carbon composition may both influence sorption of hydrophobic organic pollutants on low-carbon aquifer materials ^{38, 98}.

A sorption isotherm using phenanthrene concentrations close to saturation, revealed that the partitioning between phenanthrene and the sand was linear to an aqueous concentration of approximately 80% of saturation (Figure 8). This is consistent with the results of Karickhoff et al. ³ who observed that sorption isotherms were linear to at least 10^{-5} M or about half the aqueous solubility.

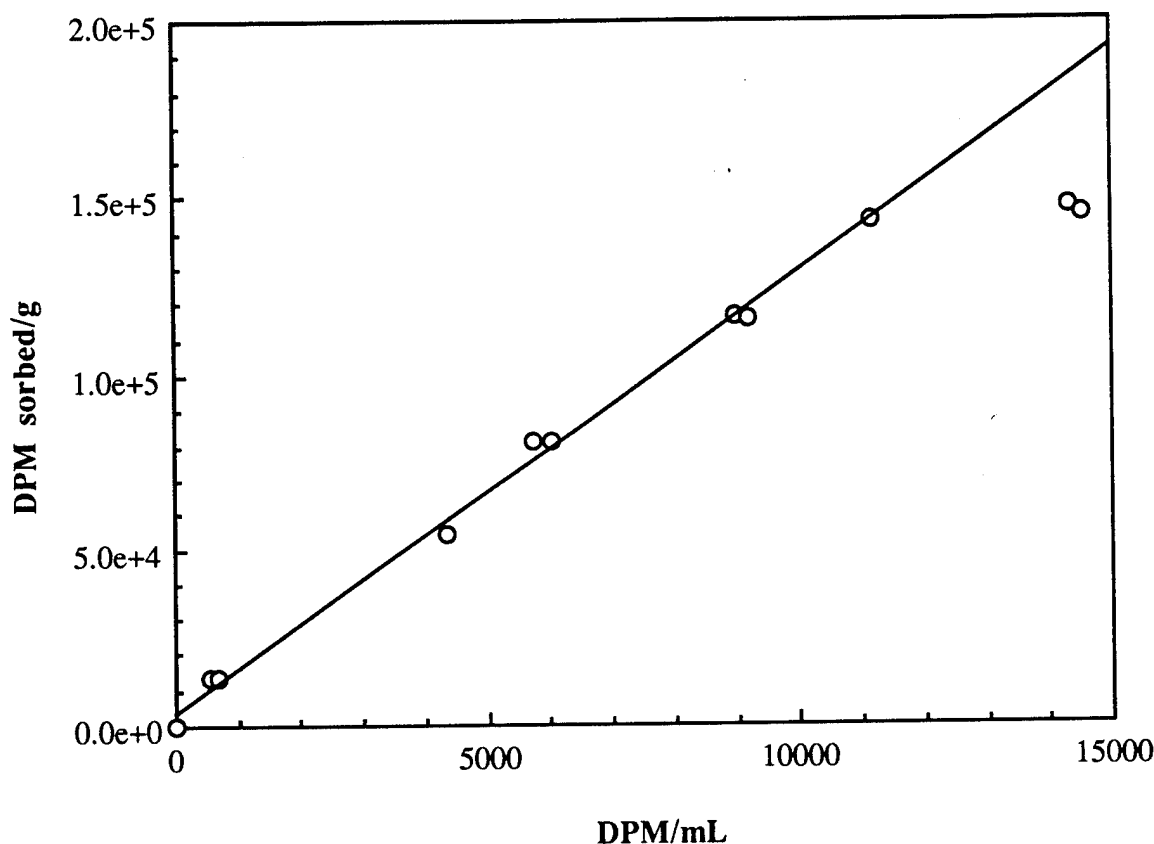


Figure 8. Phenanthrene sorption isotherm on aquifer sand with aqueous concentrations approaching the solubility limit. [Data are plotted using Equation (16).]

2. In the Presence of Polymer

Twenty-eight different microbial polymers were screened for their ability to reduce the partition coefficient of phenanthrene on the sand. Figure 9 shows an example of a batch isotherm for an extracellular polymer obtained from isolate 9702M-4, a gram negative motile rod, which had a significant influence on the partitioning of phenanthrene. By using the partition coefficient estimated from the slope of the isotherm ($K_d' = 12.6 \text{ mL/g} \pm 1.5$, $n=2$) and an average $\rho_b/n = 4.65 \text{ g/mL}$ for the sand, the retardation factor can be calculated using Equation (7). The presence of the polymer would result in a reduction of the retardation factor from 96.3 to 59.6, or 38%.

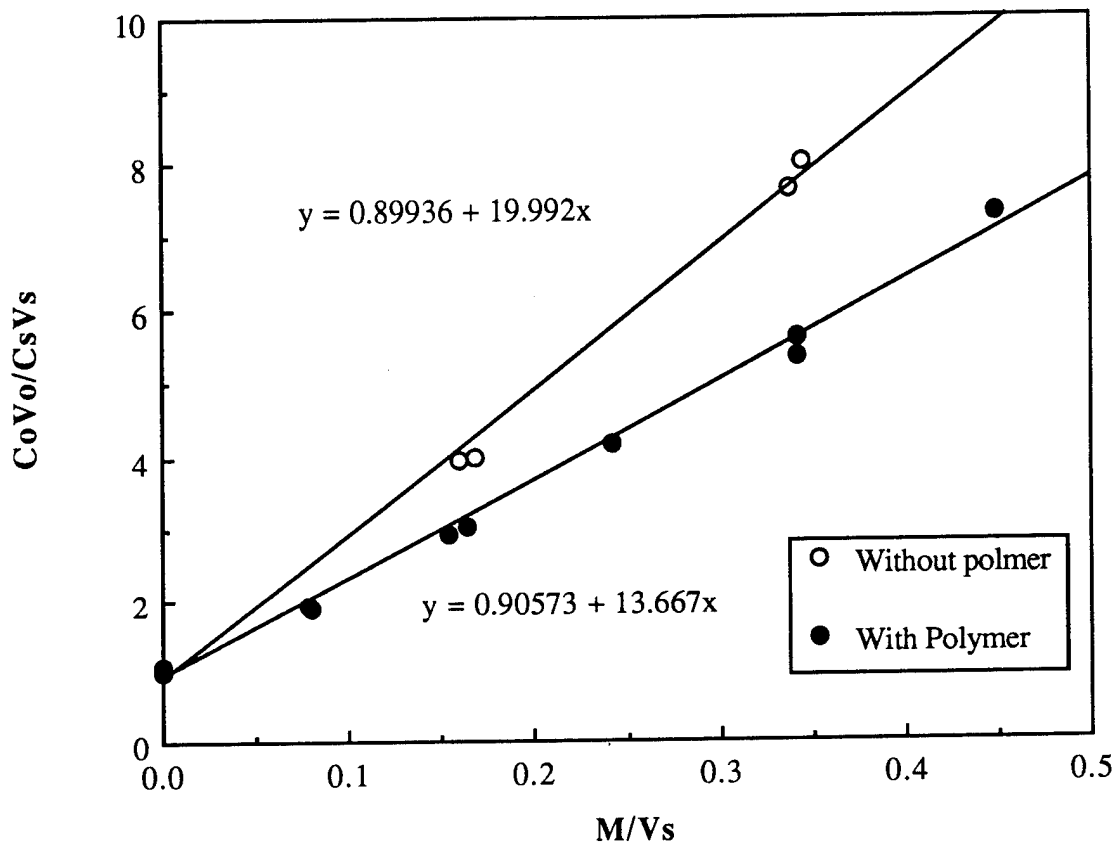


Figure 9. Phenanthrene sorption isotherm on an aquifer sand in the presence and in the absence of 100 mg TOC/L of extracellular polymers produced by soil isolate 9702M-4.

Figure 10 summarizes the results of all batch isotherms obtained from various polymers. Where feasible, both extracellular (*exo*) and cell-bound (*cap*) polymers from the same isolate were analyzed. Some polymers increased the partition coefficient (and hence the retardation factor) of phenanthrene; these polymers would theoretically further retard the transport of phenanthrene in soils. As a whole, however, most polymers tended to decrease the phenanthrene distribution coefficient. As a result of this screening procedure, the extracellular polymer produced by isolate 9702M-4 was selected for further characterization and analysis in column experiments.

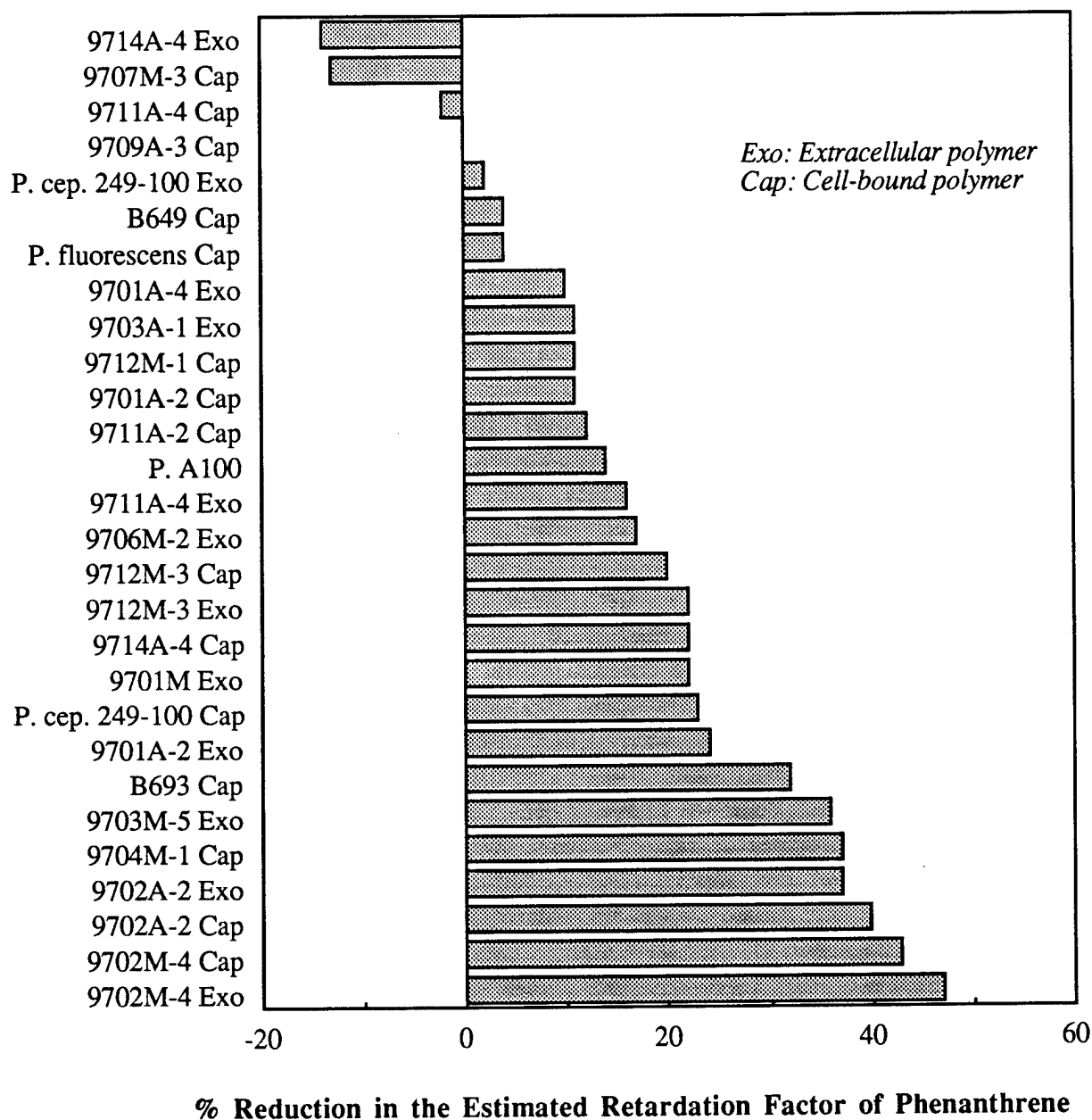


Figure 10. Summary of the predicted effects on the retardation factor of phenanthrene in an aquifer sand in the presence of 100 mg/L TOC/L of the indicated microbial polymers.

It was observed in batch experiments that samples containing polymer remained highly turbid while samples without polymer were completely clear after equal settling times. This may have been due to destabilization of colloids present in the soil by the polymer. Distribution coefficients between phenanthrene and sand in the presence of polymer obtained after 2-hour settling times were reduced an additional 35% in comparison to samples that were centrifuged. In a dynamic system, the polymer may act to mobilize existing fines and organic matter that, in turn, may act as an additional carrier for pollutants. All batch distribution coefficients given in this report are based on use of centrifugation as a solid/liquid separation procedure and therefore represent conservative estimates of the effect of polymers on PAH mobility.

C. SORPTION BETWEEN POLYMER AND SAND

A distribution coefficient of 0.926 mL/g was obtained for the exopolymer from isolate 9702M-4. This distribution coefficient is much lower than the distribution coefficient between phenanthrene and sand ($K_d = 20.5$ mL/g), thereby satisfying a criterion for possible facilitated transport (i.e., the carrier must be mobile).

D. SORPTION BETWEEN PHENANTHRENE AND EXTRA-CELLULAR POLYMER

1. Data Analysis

The measured fluorescence intensities of the phenanthrene solution were corrected for the fluorescence of the polymer alone, for dilution effects, and for what is known as the "inner filter effect." The latter is a phenomena described in detail by Gauthier et al. 89 which allows corrections for the absorption characteristics of the solution and is a function of the cell geometry and solution absorption.

The distribution coefficient for phenanthrene with the polymer can be derived in a similar manner to Equations (14-16) by equating equilibrium and mass balance equations:

$$C_T = [\text{PAH}] + [\text{PAH-Polymer}] \quad (18)$$

$$K_d^{\text{om}} = \frac{[\text{PAH-Polymer}]}{[\text{PAH}] [\text{Polymer}]} \quad (19)$$

where: C_T is the total concentration of phenanthrene in solution,

[PAH] is the aqueous concentration of phenanthrene,
 [Polymer] is the aqueous concentration of polymer,
 [PAH-Polymer] is the aqueous concentration of phenanthrene bound to polymer
 and K_d^{om} is the linear equilibrium distribution coefficient for phenanthrene with the polymer.

Combining these two equations and assuming that the fluorescence intensity, F , is proportional to the free phenanthrene in solution then:

$$\frac{F_o}{F} = \frac{C_T}{[PAH]} = 1 + K_d^{om} [Polymer] \quad (20)$$

A plot of F_o/F vs. [Polymer], known as the Stern-Volmer plot, will give a line with a slope equal to K_d^{om} .

2. Results of Fluorescence Quenching Method

Figure 11 shows the Stern-Volmer plot for phenanthrene and soil isolate 9702M-4 exopolymer with a measured K_d^{om} value of 24,350 mL/g. Since the K_d^{om} values for polymers are on an organic carbon basis, they can be directly compared to the K_{oc} value measured between phenanthrene and the soil. The measured value of K_d^{om} is comparable to Karickhoff's measurement of $K_{oc} = 22,900$ mL/g but lower than the value measured in this research ($K_{oc} = 42,200$ mL/g). Means and Wijayaratne 44 found that colloidal material was 10 to 35 times more effective as a sorbate for atrazine and linuron than was the organic carbon in soils and sediments and attributed this difference to unavailable soil organic matter held within the inorganic matrix. Indeed, aqueous colloidal organics are thought to have a greater available surface area than bound soil organics 99. However, for the experiments performed by Means and Wijayaratne, ion exchange or condensation reactions could also have caused the observed data. Gschwend and Wu suggested that the K_{oc} for colloidal matter could be less than the K_{oc} for soil organic matter since colloids in solution are more hydrophilic than soil-bound organic matter and would have less affinity for hydrophobic pollutants. Baker et al. 4 found that the ratio of the partition coefficient of pollutants to colloidal matter and pollutant to soil organic matter may differ from 0.5 to 35. Although, generalizations may not be appropriate, there may still be an optimal condition for facilitated transport given that the hydrophilic character of the colloid could be offset by the increased surface area. Mobile colloids having a hydrophobic core may be very effective carriers if they also contain hydrophilic functional groups and the mass transfer limitations of pollutants into the core are insignificant.

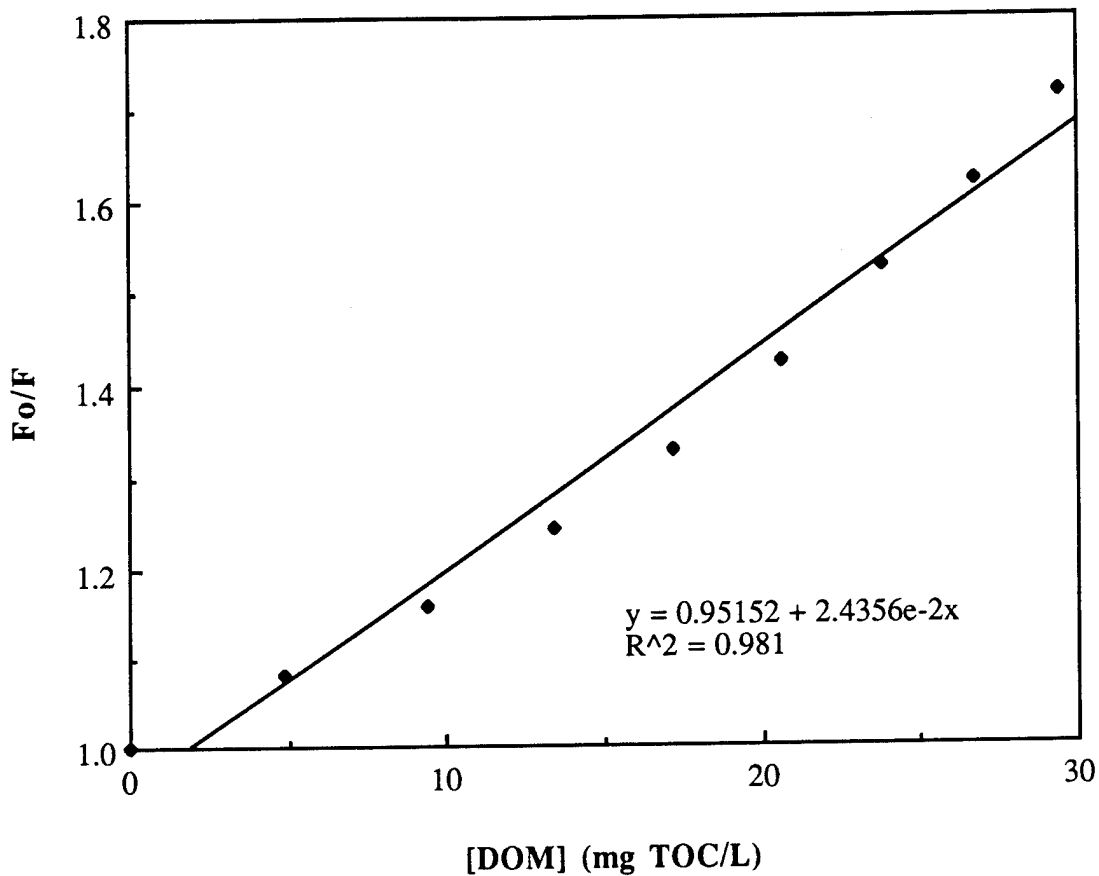


Figure 11. Stern-Volmer plot for the partitioning of phenanthrene onto the extracellular polymer produced by soil isolate 9702M-4.

E. MODEL RESULTS

Substituting the values of the three distribution coefficients obtained above and an average column ρ_p/n of 4.65 g/mL into the three component model [Equation (8)], the phenanthrene retardation factor in the presence of 100 mg C/mL of polymer was calculated to be 67.7. Using the effective partition coefficient of phenanthrene in the presence of polymer, $K_d' = 12.6$ mL/g, and Equation (7), the predicted retardation factor would be 59.6. These values are in good agreement and support the validity of the three-component model developed by Magee et al. ⁴¹.

F. RESULTS OF COLUMN STUDIES

1. Data Analysis

Column breakthrough curves (BTCs) are an attempt to simulate subsurface groundwater flow and were performed to compare the retardation of a compound with that predicted based on batch isotherm results.

The retardation of a compound can be determined from column experiments by either frontal analysis ¹⁰⁰, or the first temporal moment of the breakthrough curve ¹⁰¹. Frontal analysis determines the total amount of water displaced until $C/C_o = 1.0$ while the temporal moment estimates the mean breakthrough time. Using frontal analysis, the retardation factor can be calculated by the equation:

$$R = \int_0^{\theta_o} \left(1 - \frac{C}{C_o}\right) d\theta \quad (21)$$

where: θ_o is the total pore volumes when $C/C_o = 1$.

A prerequisite for using Equation (21) is that a C/C_o value of 1 must be achieved, which may require large amounts of the compound of interest and may be difficult to obtain for hydrophobic compounds. Alternatively, the first temporal moment can be calculated from the measured data using:

$$R = \frac{\int_0^{\infty} \frac{C}{C_o} \theta d\theta}{\int_0^{\infty} \frac{C}{C_o} d\theta} - \frac{\theta_p}{2} \quad (22)$$

where: θ_p is the pulse width.

Equation (22) allows flexibility with regard to the amount of pollutant used in the pulse input and the maximum C/C_o value that is observed, but may prove difficult to interpret if excessive tailing of the BTC occurs. Both Equations (21) and (22) are independent of sorption kinetics although nonequilibrium will effect the shape of the breakthrough curve ¹⁰¹.

2. Chloride Breakthrough

To estimate the pore water velocity and dispersion coefficient within the column, a chloride pulse was added as an inert tracer with an assumed retardation of 1. The column data was fit using a least-squares regression of the advection-dispersion equation using the program CXTFIT 93. Figure 12 shows a chloride BTC along with the least squares regression. The chloride BTCs were not completely symmetrical which is probably a result of hydrodynamic effects such as the presence of dead end pores rather than kinetic limitations ³³. Retardation as determined by frontal analysis and first temporal moment of the chloride BTC both revealed a retardation coefficient of approximately 1.0. The fitted values for the average pore velocity and the dispersion coefficient for the glass columns packed with the aquifer sand were 7.22 ± 0.53 cm/hour and 3.26 ± 0.91 cm²/hour ($n=5$), respectively. Estimations from bulk flow rate and cross sectional area confirmed the result for the pore water velocity.

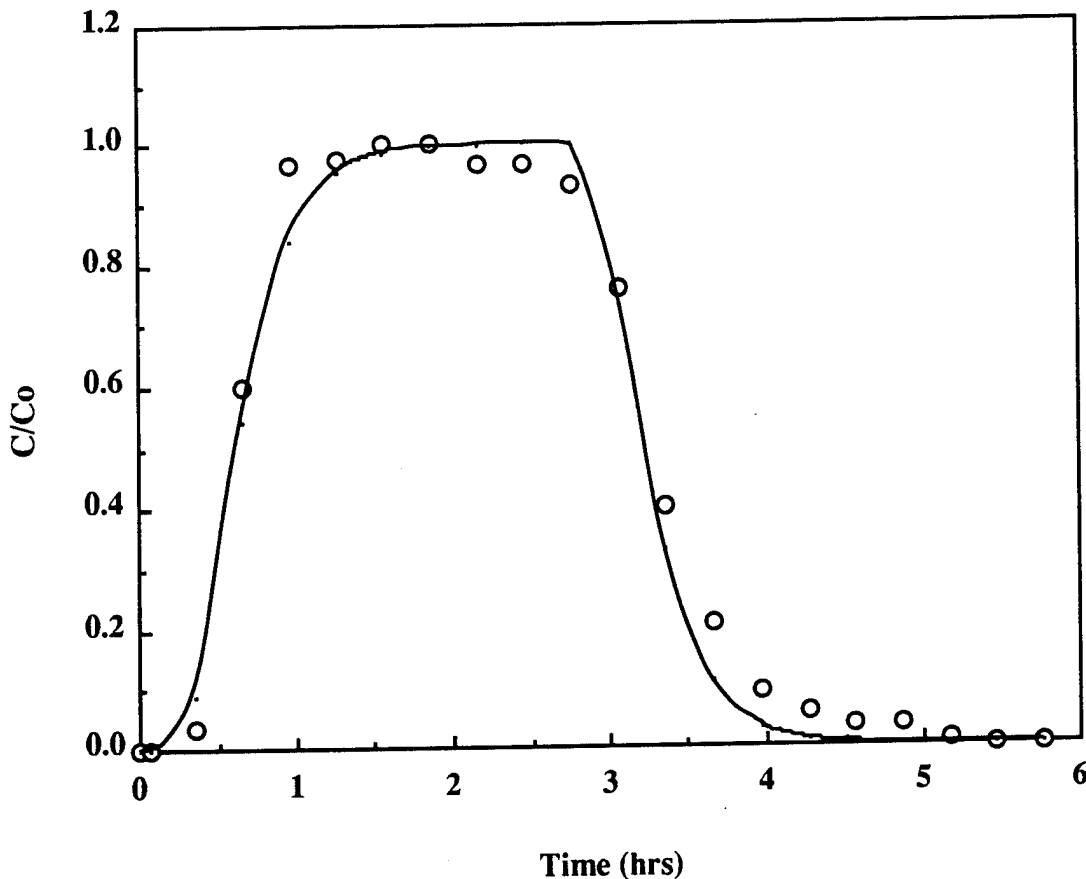


Figure 12. Column breakthrough of chloride and the least squares fit calculated from CXTFIT 93.

3. Polymer Breakthrough

The extracellular polymer produced by isolate 9702M-4 exhibited limited retardation relative to chloride but a portion of the polymer input mass was "irreversibly" retained or filtered (Figure 13). Slight tailing was apparent and has been observed in other studies for dissolved organic carbon (DOC) ¹⁰². The retardation factor was determined to be approximately 1.5 from the first temporal moment [Equation (22)] which compared to a value of 5.3 as estimated by the batch partition coefficient ($K_d = 0.926 \text{ mL/g}$ and $\rho_b/n = 4.65 \text{ g/mL}$). This difference is similar to the results observed by Jardine et al. ¹⁰³ for the transport of DOC through an aquifer sediment.

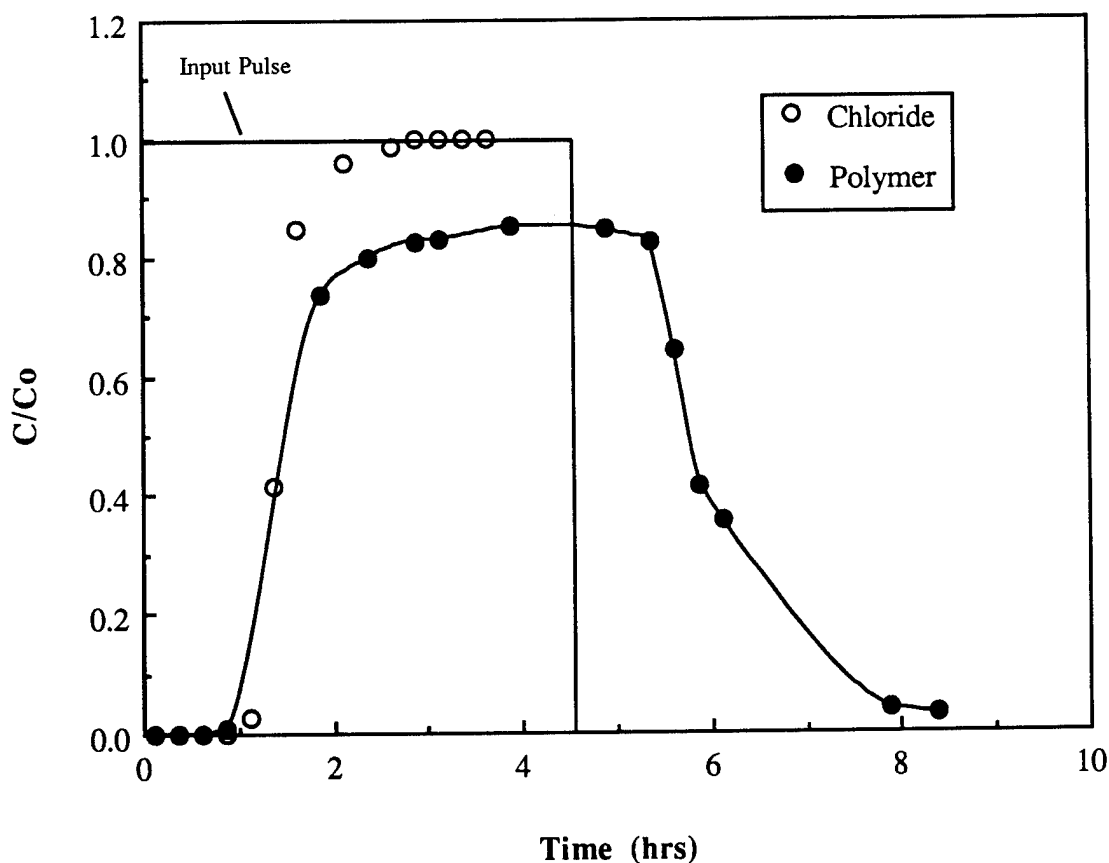


Figure 13. Breakthrough curve of extracellular polymer produced by soil isolate 9702M-4.

Jardine et al. ³⁷ found that sorption of dissolved organic carbon (DOC) was highly dependent on solution pH with a maximum at 4.5. The pH for the column system in the research reported here was 6.5 and was nearly identical to the pH in the batch system. Large amounts of indigenous soil organic matter tended to reduce the retardation of added DOC ³⁷. Iron oxides and hydroxides were determined to account for up to 70% of the total DOC adsorbed. For a low-carbon, low-iron content sand with a circumneutral pore water pH, there would be presumably low adsorption of the polymer to the soil matrix. These properties of the sand matrix may, therefore, represent optimal conditions for the transport of the polymer through the soil. If the polymer acts as a "carrier," then increased "carrier" transport would result in increased pollutant transport.

4. Phenanthrene Breakthrough in the Absence of Polymer

Breakthrough curves of phenanthrene revealed considerable tailing (Figure 14). This may be a result, in part, of differences in molecular diffusion coefficients between chloride and phenanthrene that result from variations in molecular size. If this were the case, however, the BTC of the macromolecular extracellular polymer (see Figure 13), with a molecular weight equal to $\approx 500,000$, would have exhibited significant tailing relative to the phenanthrene BTC. The lack of significant tailing in the polymer BTC would therefore lead to the conclusion that the tailing in the phenanthrene BTC is a result of kinetic limitations in the sorption process. Mass recovery averaged around 80% after 200 pore volumes making estimates of the mean breakthrough time difficult. It was observed, that the tail of the BTC data fit a curve of the form:

$$\frac{C}{C_0} = a \theta^{-b} \quad (23)$$

where: a and b are constants ($r^2 > 0.99$).

Extrapolating the data to 2000 pore volumes accounted for 90% of the input mass. Although the estimated C/C_0 values become very small at high pore volume values, the temporal moment is weighted heavily by values in the tail. Since Equation (23) will never actually reach zero, the retardation factor using the extrapolated data will continue to increase depending on the length of the extrapolation period.

The average retardation factor (based on recovery of 80% of the input mass) as determined by Equation (22) was 43.5 ± 2.1 ($n=2$) which was less than half the value as estimated by the batch isotherm partition coefficient ($R = 96.3$) [Equation

(7)]. As noted above, failure to recover input mass in the tail of the BTC would account for the lower R value in the column experiments.

5. Phenanthrene Breakthrough in the Presence of Polymer

Breakthrough curves of phenanthrene in the presence of an extracellular polymer produced by isolate 9702M-4 had higher (by approximately 10%) peaks as well as a better mass recovery than the breakthrough curves without polymer (Figure 14). Phenanthrene also appeared in the column effluent at an earlier point in the breakthrough curve. These results support the theory that phenanthrene in the presence of polymer is more mobile than in its absence. The retardation factor calculated from the BTC (based on 80% mass recovery), revealed $R = 26.5 \pm 2.0$, a 39% reduction compared to the BTC without polymer. Comparing the retardation factor values using the amount eluted after 150 pore volumes, gave 40.5 ± 2.2 for the BTCs without polymer and 33.9 ± 3.4 for the BTCs with polymer with recovery rates of $76.5 \pm 2.3\%$ and $87.9 \pm 1.9\%$, respectively. Due to the variable amounts of mass recovered in each column experiment, a quantification of the effect of polymer on the retardation factor becomes difficult to interpret. It is evident, however, that the presence of the polymer increased the mobility of phenanthrene in the aquifer material. Fitting the tail of the phenanthrene / polymer BTC data with the regression equation [Equation (23), $r^2 > 0.99$] resulted in essentially complete mass recovery (at roughly 600 pore volumes).

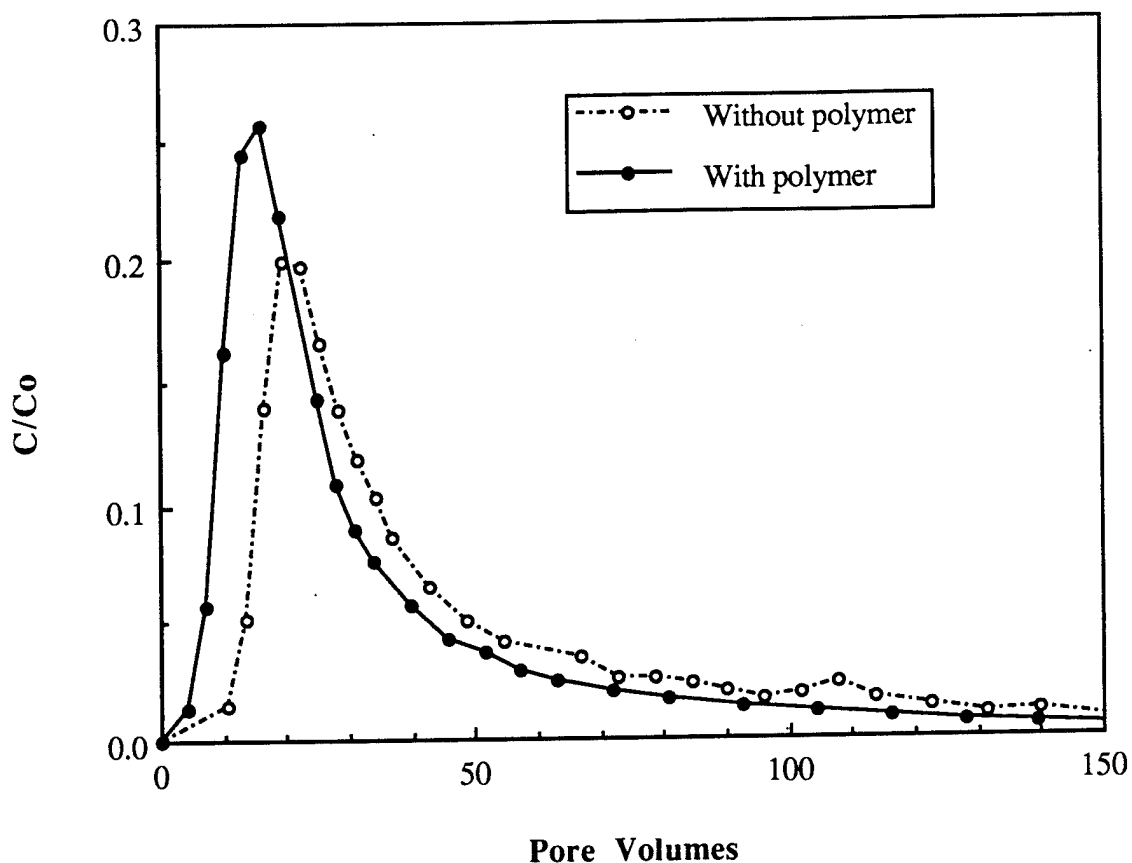


Figure 14. Phenanthrene breakthrough curves in the presence and in the absence of the extracellular polymer produced by soil isolate 9702M-4.

6. Comparison with Model Calculations

As previously noted, the significant tailing observed and lack of full mass recovery make it difficult to compare the measured column retardation factors with the retardation factors predicted by the batch isotherm and the three-component model. Other factors (discussed below) may also have contributed to differences, such as (1) the removal of fines in the column, (2) the failure to achieve equilibrium in batch experiments, (3) the existence of a solids-effect in the batch experiments and (4) a nonlinear sorption isotherm.

Particle analysis of the column effluent revealed that fines were removed from the sand during the initial stages of flushing the column. A batch isotherm experiment using sand that had been flushed, however, revealed no reduction in the phenanthrene partition coefficient. Therefore, the fines removed from the column were not responsible for the disparity in predicted and measured retardation factor values.

The distribution coefficient obtained from batch experiments was based on an operational definition of "dissolved" compounds, i.e. all mass remaining in solution after centrifugation. Gschwend and Wu ⁹⁹ have reported that some colloidal solids may not be removed. The resulting error is termed the "solids effect" which will lead to an underestimation of the true partition coefficient. This effect would, however, cause the difference between batch and column retardation factors to become even greater. A failure to achieve equilibrium in batch experiments would also result in an underestimation of the batch distribution coefficient.

The phenanthrene sorption isotherm was nonlinear at the highest concentration tested (Figure 8). For all the column experiments reported, however, influent concentrations were well within the linear range and hence cannot be responsible for the observed differences in batch and column retardation values.

Given the above observations, it can be concluded that batch isotherms are a good qualitative method for comparing the transport of a compound with varying environmental parameters but quantitative prediction of column behavior based on batch results may prove difficult.

7. CXTFIT Calculations

The phenanthrene BTCs show apparent kinetic limitations due to the high amount of tailing observed. CXTFIT ⁹³ can be used to estimate kinetic parameters by assuming that only a fraction of the sites are at equilibrium (F) while the remaining sites are subjected to a first order rate coefficient α . The BTC data were fit using two free parameters, one related to F and the other to α . Input parameters were the pore water velocity, the pore water dispersion coefficient (both determined from a fit to the chloride BTC), the retardation factor (determined from batch partition coefficient), and the input pulse width. An additional data fit was carried out allowing the model to solve for the retardation factor in addition to the other two adjustable parameters. The BTC predicted by the parameters generated by CXTFIT adequately described the peak in the phenanthrene BTC but overestimated the tail (Figure 15). A summary of the model results is listed in Table 7.

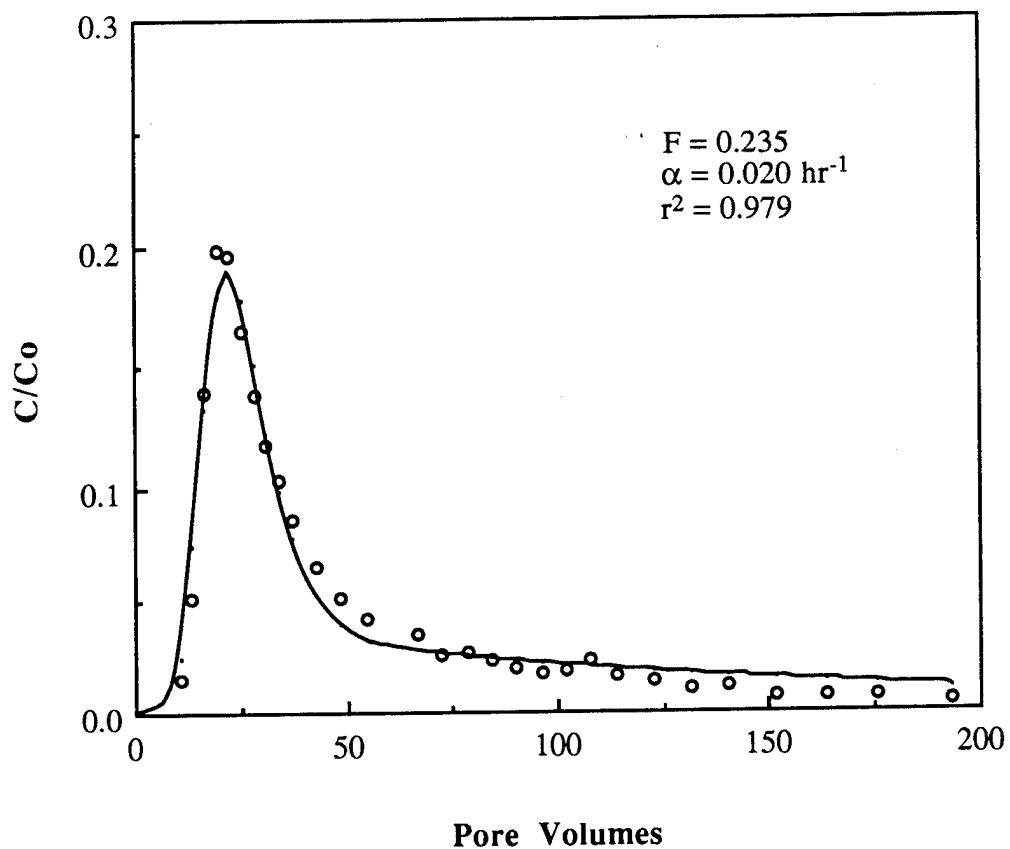


Figure 15. Typical phenanthrene BTC in the absence of polymer and comparison to a fit of the data to the two site/two region model generated using CXTFIT 93.

Table 7. Results of Two-site/Two-region Model fits for Phenanthrene Breakthrough Curves.

Fixed R:

<u>Polymer</u>	<u>R</u>	<u>F</u>	<u>$\alpha(\text{hr}^{-1})$</u>	<u>r^2</u>
no	96.3	0.235	0.020	0.979
no	96.3	0.190	0.016	0.966
yes	59.6	0.279	0.037	0.979
yes	59.6	0.236	0.033	0.973

Free R:

no	85.5	0.270	0.025	0.983
no	69.2	0.267	0.028	0.981
yes	47.2	0.335	0.055	0.990
yes	47.7	0.288	0.047	0.981

The first-order rate coefficient for the two-site model can also be estimated through an empirical relationship ($r^2 = 0.95$) developed by Brusseau and Rao 104:

$$\alpha = 2 (K_d)^{-0.668} \quad (24)$$

Using the measured partition coefficient from batch isotherm experiments, an estimated rate coefficient of $\alpha = 0.262 \text{ hr}^{-1}$ was obtained. This value compares to an average estimated CXTFIT value of 0.018 hr^{-1} . The two-site model may be oversimplified by defining sorption as governed by a single rate constant and a fraction of sites that obey local equilibrium. Connaughton et al. 105 found better fits to data for the desorption of naphthalene from soils by assuming a distribution of first order rate constants, rather than one distinct rate. Given the often complex composition of natural soils 34, 36, the existence of several sorption sites with differing kinetic rate constants seems a plausible explanation.

The inability to model the column breakthrough curve data accurately from independently measured or estimated parameters such as R and α , leads to the conclusion that the present understanding of hydrophobic pollutants in even simple, well-defined, systems is deficient.

G. EXTRACELLULAR POLYMER CHARACTERIZATION

1. Size Exclusion Chromatography

Microbial polymers are complex structures, the size of which can vary greatly depending on chain length and the amount of branching, even within polymers produced by the same bacterial species. Sizes can range from 4×10^4 to over 5×10^7 daltons with sizes varying depending on the strain and the culture conditions used ⁵⁷.

Table 8 lists the molecular weight standards used to create a HPLC calibration curve (Figure 16) and the chromatographic elution times of the standards and selected polymers. Several smaller molecular weight impurities were evident in the chromatogram in the polymer from isolate 9702M-4 ¹⁰⁶ but the predominant component had a molecular weight of approximately 500,000 daltons. The polymers from the other isolates were of slightly higher molecular weights, ranging up to 600,000 daltons for the extracellular polymer from isolate 9702A-2. The molecular weights of both the cell-bound and the extracellular polymer from isolate 9701A-2 were on the same order of magnitude. As shown in Figure 10, the polymer from isolate 9701A-2 was substantially less effective at mobilizing phenanthrene than the polymer from isolates 9702M-4 or 9702A-2. No apparent relationship was observed between a polymers molecular weight and its ability to enhance the transport of phenanthrene.

Table 8. Elution Times for Standards and Selected Polymers.

<u>Compound</u>	<u>Molecular Weight</u>	<u>Elution Time (min)</u>
Albumin (bovine)	66,000	6.67
Alcohol dehydrogenase	150,000	6.49
Amylase	200,000	6.22
Apo ferritin	443,000	5.99
Thyroglobulin	669,000	5.95
Blue Dextran	2,000,000	5.61
<i>estimated from regression [†]</i>		
9702M-4 Exo polymer	520,000	6.01
9701A-2 Exo polymer	575,000	5.97
9701A-2 Cap polymer	545,000	5.99
9702A-2 Exo polymer	600,000	5.95

[†]Exo: extracellular polymer, Cap: cell-bound (capsular) polymer

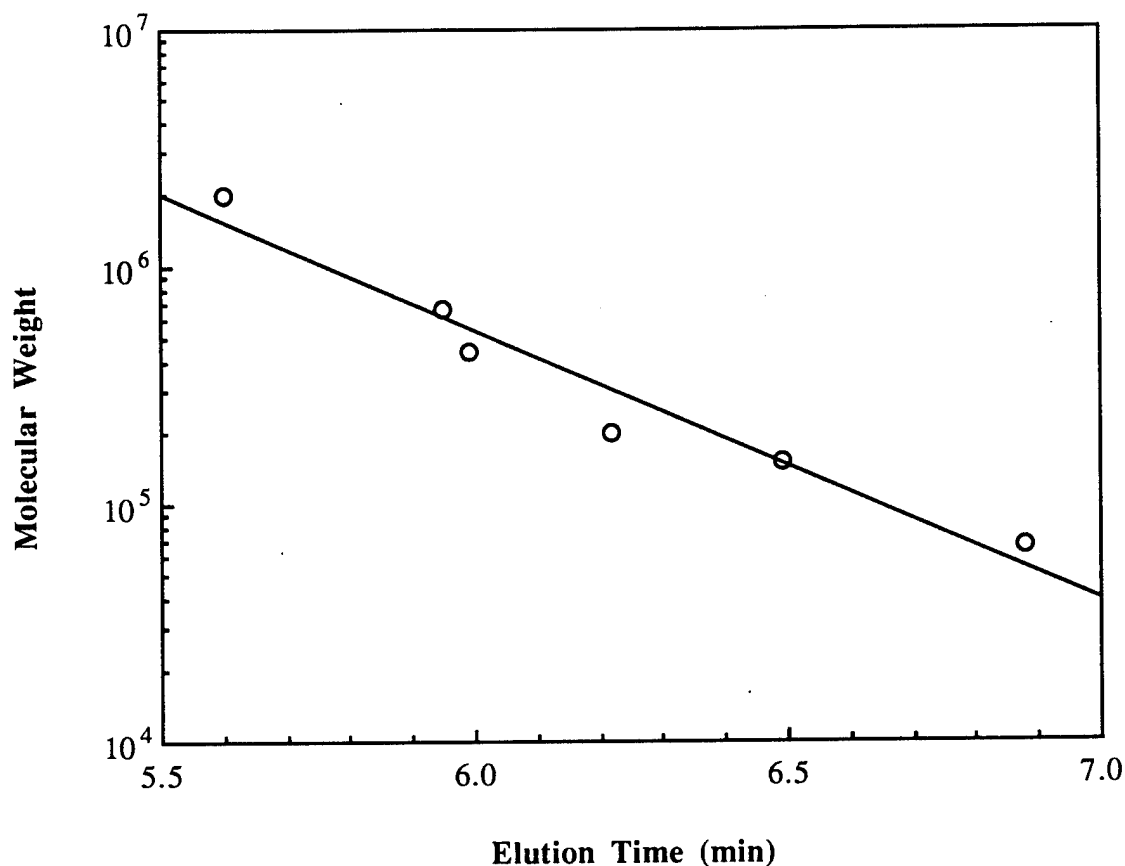


Figure 16. HPLC calibration curve used to estimate the molecular weight of microbial polymers.

2. IR Spectroscopy

Exopolysaccharides can vary in structure as much as they do in size. They often consist of long branched chains with repeating components and functional groups. Some components such as D-glucose, D-mannose, D-galactose and D-glucuronic acid are commonly found in most microbial polymers. Other components such as L-rhamnose and L-fucose are less frequent ⁵⁷. Functional groups such as amides or carboxyl groups may be indicative to the structure and behavior of the polymer in an aqueous environment.

The spectra of several polymers were obtained to test for a correlation between sorptive behavior and the presence of specific functional groups. All the

spectra obtained had a wide band at 3440 cm^{-1} , that corresponds to the O-H stretch commonly found in sugars and nonaromatic alcohols 107. All extracellular polymers also revealed IR bands at 2920 cm^{-1} , which is characteristic of the C-H stretch of CH_2 and CH_3 groups. None of the cell bound polymers that were tested contained such a band suggesting compositional variation between cell-bound (capsule) and extracellular polymers. Table 9 summarizes the IR peaks for selected polymers as well as D-glucose 107. The other common sugars that are often components of microbial polysaccharides have similar IR peaks to those for D-glucose. As can be seen, many of the functional peaks of the polymers have corresponding peaks in the spectra of D-glucose, suggesting a polysaccharide composition. However, no immediate correlation between functional groups, or combinations thereof, and effect of a polymer on phenanthrene retardation was apparent from the data obtained and no conclusions could be made regarding the effect of specific functional groups.

Table 9. Prominent Features of the IR Spectra for Selected Extracellular Polymers

Wavenumber 1/cm	9701A-2 Exo ^a	9702A-2 Exo	9702M-4 Exo	A 100 Exo	9701A-2 Cap ^a	9707M-3 Cap	D- glucose	Functional Group ^c
3400	x	x	x	x	x	x	x	O-H stretch ^d
2920	x	x	x	x			x	C-H stretch of CH_n
1725			x	x				C=O of acids
1640		x				x		C=O of amide
1625	x		x	x	x			Aromatic C=C
1330					x	x		Aromatic amine
1195	x	x		x			x	O-H bend
1125	x	x		x	x		x	unknown
1075			x		x			C-N stretch
915						x	x	C-H of alkenes
600		x		x				N-H bend
%R ^b	24	24	50	17	11	-9		

a Cap: cell-bound (capsular) polymer, Exo: extracellular (slime) polymer

b %R: % change in the retardation factor of phenanthrene in the presence of the polymer

c Silverstein et al. 108

d Pouchert 107

3. Polymer Production

The isolate 9702M-4 was analyzed for cell growth and polymer production. Optical density measured at 600 nm and polymer production measured by organic carbon were directly proportional to cell mass. The bulk of the polymer was produced in the early exponential phase but some production seemed to occur in the stationary phase (Figure 17). This result was consistent with those of Hsieh ¹⁰⁹ who observed that biopolymer accumulation occurred through both growth and nongrowth processes and was proportional to active cell mass. No distinction was made between compositional variations in the polymers produced during the various stages of growth.

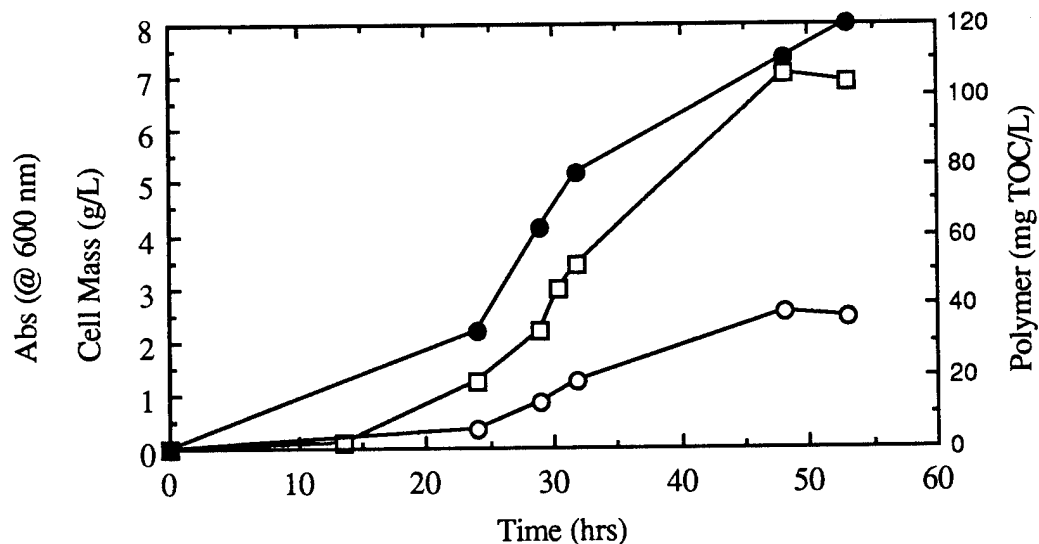


Figure 17. Production of extracellular polymer from bacterial isolate 9702M-4 (●) as a function of cell mass (○) and optical density of the culture broth (□)

H. PHENANTHRENE MINERALIZATION

1. Mineralization in an Aqueous System Without Sorbent

The presence of the polymer seemed to increase the extent of mineralization of phenanthrene in a system without a sorbent (Figure 18). Loss of ^{14}C -phenanthrene through or onto the septa may have occurred as mass recovery rates averaged $69.7 \pm 0.5\%$ for samples without polymer and $72.6 \pm 0.4\%$ for samples with polymer. The small difference in total recovery, however, would indicate that different rates of volatilization are not responsible for the observed difference. This result indicates that the presence of this particular polymer was beneficial with regard to the mineralization of phenanthrene in an aqueous system.

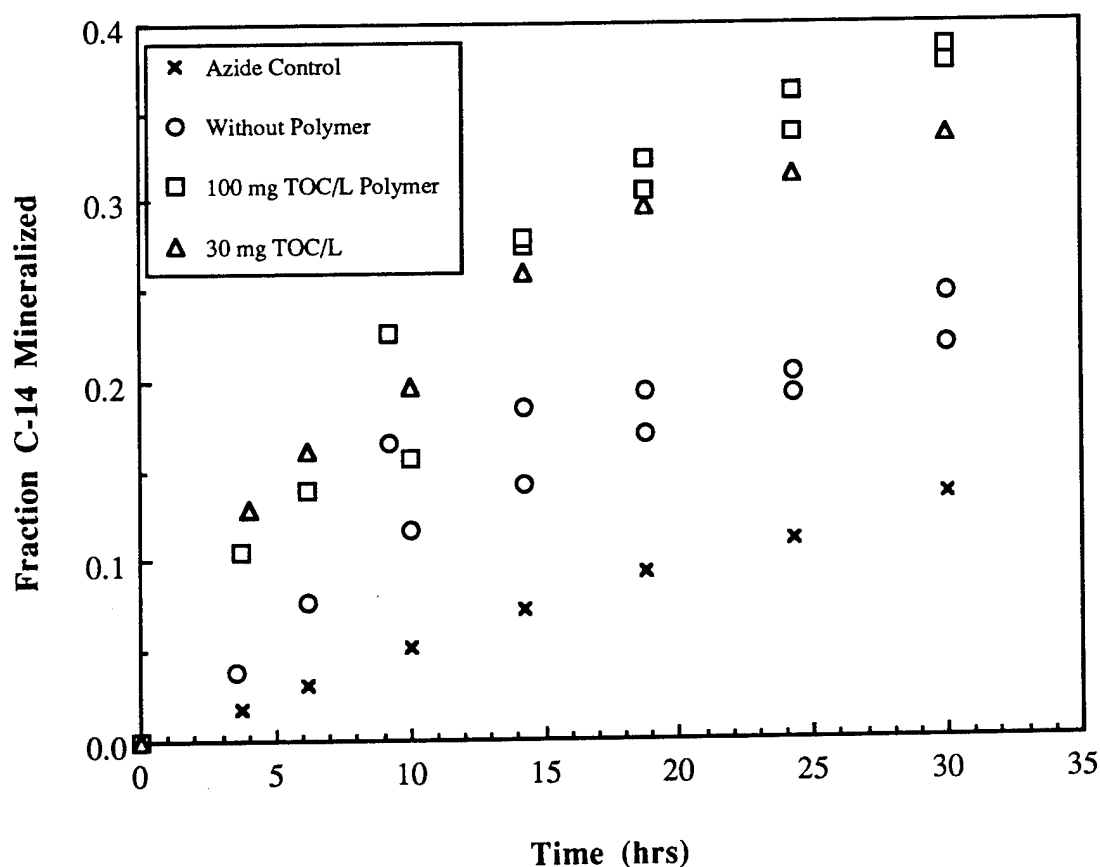


Figure 18. Effect of dissolved extracellular polymer on the mineralization of dissolved phenanthrene (in the absence of a sorbent) by a mixed bacterial culture.

2. Mineralization in an Aqueous System with Sorbent

Phenanthrene mineralization experiments in the presence of the low-carbon aquifer sand indicated that the presence of the extracellular polymer from isolate 9702M-4 had little or no effect on mineralization (Figure 19). These results suggest that the rate of phenanthrene degradation may be limited by the desorption kinetics of phenanthrene from the sand.

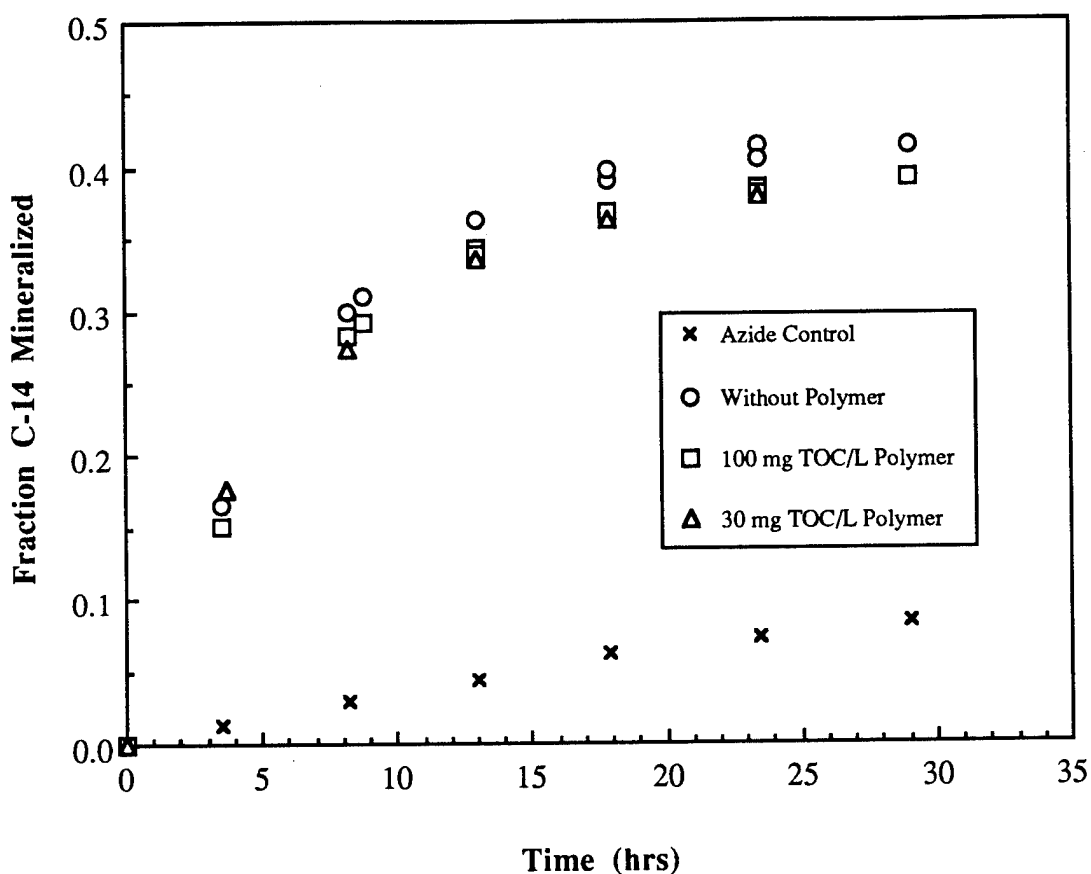


Figure 19. Effect of dissolved extracellular polymer on the mineralization of phenanthrene (in the presence of a sorbent) by a mixed bacterial culture.

Rapid degradation of the polymer before or during the onset of phenanthrene mineralization could also explain the results of phenanthrene mineralization in the presence of the sand. The degradation characteristics of microbial polymers vary from one isolate to the next. Williams and Wimpenney⁶⁵ observed no degradation of an extracellular polymer produced by a *Pseudomonas* species while

Bodie et al. 110 observed degradation of an extracellular polymer produced by an *Arthrobacter* species.

Another (untested) possibility is that enzymatic activity of the mixed culture inoculum may have hydrolyzed the polymer into smaller components that had limited binding capacity for phenanthrene. This would be an undesirable effect if it occurred since it would reduce the ability of the polymer to act as a "carrier" in the field.

I. BATCH EXPERIMENTS WITH WHOLE BACTERIAL CELLS

1. Phenanthrene Sorption to Bacterial Isolates:

The batch isotherms for the bacterial isolates tested were linear, and significant differences between organisms were evident (Table 10). The K_d^{cell} values were lower than the K_{oc} value for phenanthrene with the aquifer sand (average sand $K_{oc} = 5.3 \times 10^4$ mL/ g organic C). As a group, the K_d^{cell} values for the methanotrophic strains were at the high end of the range of observed values.

Table 10. Partition Coefficients for Phenanthrene with Bacterial Cells.

Bacterial isolate	K_d^{cell} (mL/g cell carbon) +/- S_d ($\times 10^3$)*
<i>Acinetobacter calcoaceticus</i> 31012	5.6 +/- 0.1
<i>Pseudomonas cepacia</i> 249-100	8.1 +/- 0.3
<i>P. fluorescens</i> 13524	8.4 +/- 0.2
A100	13.2 +/- 0.3
B121	10.5 +/- 0.3
B550	7.3 +/- 0.2
B649	7.9 +/- 0.2
B693	6.2 +/- 0.1
9702A-2	11.1 +/- 0.1
9703A-1	3.0 +/- .03
9703A-5	3.3 +/- 0.1
9710M-3	10.0 +/- 0.2
<i>M. trichosporium</i> OB3b	16.8 +/- 0.6
<i>M. parvis</i> OBBP	21.5 +/- 1.0
<i>M. albus</i> BG8	11.3 +/- 0.2

*No r^2 value for the linear isotherms was less than 0.940

The presence of suspended bacteria at cells densities $>100 \mu\text{g TOC mL}^{-1}$, in all cases but one ($n = 23$), decreased the value of the distribution coefficient for phenanthrene with the aquifer sand. Most isolates (74%) at concentrations between 20 and $55 \mu\text{g TOC mL}^{-1}$ significantly decreased ($>10\%$) the K_d value for phenanthrene with the aquifer sand as measured by the level of radioactivity in suspension after settling of the sand (Table 11).

Table 11. Reduction of K_d Value for Phenanthrene with the Aquifer Sand.

Bacterial isolate	minimum* $\mu\text{g TOC/mL}$	% reduction
<i>Arthrobacter globiformis</i>	45	<5
<i>Acinetobacter calcoaceticus</i>	43	<5
<i>Bacillus subtilis</i>	52	25
<i>Pseudomonas cepacia</i> 249-100	22	21
<i>Pseudomonas fluorescens</i> 13524	49	15
A100	44	25
B649	20	10
B693	48	<5
9701A-2	50	47
9702A-2	55	30
9702M-4	58	31
9703A-1	38	37
9706M-2	37	<5
9707M-3	45	<5
9709A-3	40	29
9711A-2	26	43
9711A-4	49	<5
9712M-1	46	20
9712M-3	37	27
9714A-4	29	13
<i>M. trichosporium</i> OB3b	25	25
<i>M. parvis</i> OBBP	55	39
<i>M. albus</i> BG8	39	38

*minimum concentration of cells used in the batch sorption isotherm experiments for each isolate.

A modified hyperbolic equation was fit to the data for K_d with respect to cell concentration. Because the data appeared to be a mirror image of a hyperbolic relationship (such as the Langmuir isotherm) which approaches a maximum, the equation was written to approach a minimum thus:

$$K_d = A - Ax/(B + x) \quad (25)$$

which, when rearranged, is:

$$K_d = AB/(B + x) \quad (26)$$

where A and B are adjustable parameters and x is the variable cell concentration.

Equation (26) was linearized, and the parameters were initially estimated. Using the Gaussian method ¹¹¹, the nonlinear parameters in this model were further estimated to obtain the best fit as determined by the least residual sum of squares (RSS). The fit of this equation is illustrated for isolate 9711A-2 (Figure 20). At a cell concentration of 65 $\mu\text{g TOC mL}^{-1}$, 40% of these isolates reduced the estimated K_d value by more than 30% (Table 12).

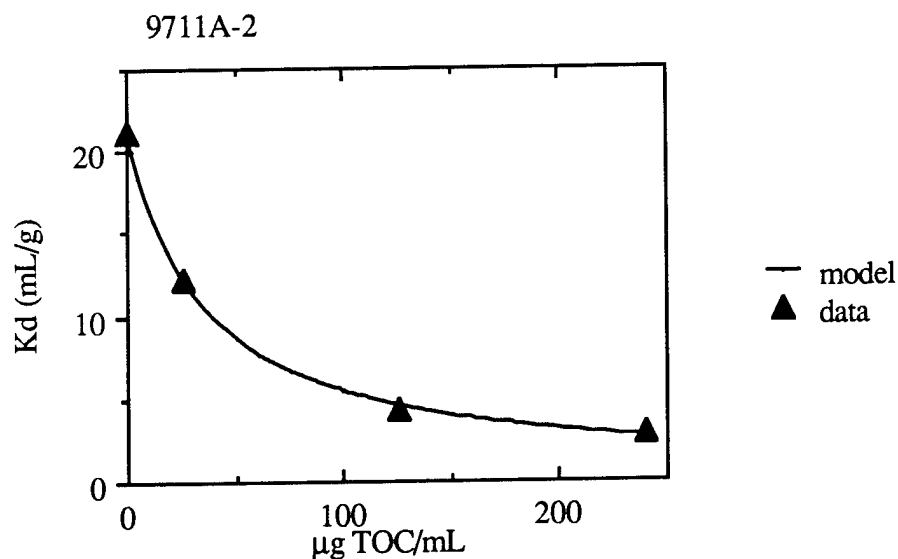


Figure 20. Effect of cell concentration on the apparent K_d value for phenanthrene with an aquifer sand. (Results for soil isolate 9711A-2.) [Model parameters: $A = 21.2$, $B = 35.0$, $\text{RSS} = 0.06$].

Table 12. Bacterial Isolates that most Effectively Decreased the Sorption of Phenanthrene onto an Aquifer Sand*

Isolate	% decrease in K_d
9701A-2	48
9702A-2	46
9702M-4	40
9703A-1	57
9709A-3	38
9711A-2	65
9712M-3	40
A100	34
BG8	50
OBBP	37

*Observed values of K_d over a range of cell concentrations were fit by nonlinear regression to a hyperbolic equation of the form $K_d = AB/(B + [\text{cell}])$. The K_d value was calculated for a cell concentration of $65 \mu\text{g C mL}^{-1}$ for purposes of comparison.

Sorption of phenanthrene by bacterial cells was observed in this research and has been reported by other investigators ²². Hydrophobic contaminants such as hexachlorobenzene (HCB), and dichlorodiphenyltrichloroethane (DDT) are also known to sorb to cells ²¹. In contrast Bellin and Rao ¹¹² recently reported negligible sorption of naphthalene by a bacterial biomass of $\sim 10^7$ cells g soil⁻¹. It is important for purposes of prediction of pollutant transport to be acquainted with the distribution coefficients for specific pollutants, such as phenanthrene, and with a spectrum of bacterial types, including those isolated from soil and subsurface environments ²¹. The data in Tables 11 and 12 contribute to this scant data base.

Steen and Karickhoff ²² showed differences in K_d^{cell} values between several mixed cultures, but not individual isolates. Other researchers have shown such differences in sorption to selected bacterial strains for environmental pollutants such as DDT ^{21, 23, 113, 114}. Previously reported K_{oc} values for phenanthrene with organic matter derived from soils ⁴¹ ($K_{oc} = 43,000$) and sediments ³ ($K_{oc} = 22,900$) were greater than the K_{oc} for phenanthrene with cells ($K_{oc} = 3000$ to $21,500$) observed in this research. The disparity in values of the distribution coefficients for phenanthrene with bacterial cells and soil organic matter may indicate a difference between these two potential carriers of nonpolar pollutants. Similarly, Chin and Gschwend ¹¹⁵ noted

significant differences in the $K_{oc}^{colloid}$ values for pyrene with organic colloids from different sediments. This observation indicates that generalizations regarding various colloidal carriers including cells may lead to erroneous conclusions. The fact that 74% of the bacterial isolates tested significantly reduced the value of the distribution coefficient for phenanthrene with the aquifer sand is indicative of the potential of bacterial cells to act as carriers of nonionic organic pollutants as depicted by McCarthy and Zachara ¹¹⁶.

J. SORPTION EXPERIMENTS WITH EXOPOLYMER FROM METHANOTROPHIC BACTERIA

The synthesis of extracellular polymer by methanotrophs has been reported; and their polymers have been shown to consist of heteropolysaccharides ^{117, 118}. With the exception of strain OBBP, the extracellular polymers of the three methanotrophs decreased the K_d value for phenanthrene with an aquifer sand (Table 13). Polymer production for these methanotrophs, under the growth conditions described, was estimated to be in the range of 20 to 30 mg L⁻¹ of cell suspension at OD₆₀₀ of 1 to 1.2. The type I strain, BG8, synthesized the largest quantities of exopolymer..

Table 13. Effect of Extracellular Polymer from Methanotrophic Bacteria on the Distribution Coefficient (K_d) For Phenanthrene.

Bacteria	[polymer] mg TOC/L	% reduction
BG8	10.6	12
OB3b	15.9	20
OBBP	6.6	<5

TOC analysis indicated that carbon made up only 10 to 12% of the total mass of the polymers produced by the methanotrophic cell strains. This low percentage of organic C was enigmatic and analysis of methanotrophic polymer composition continues as the subject of ongoing research. The sorption isotherm data for the three extracellular polymers tested has indicated that the effect of polymers from the methanotrophic isolates OB3b and BG8 on PAH transport may be significant. Further research on the extracellular polymers produced by methanotrophic bacteria appears to be merited.

K. BACTERIAL CELL MOBILITY

Five subsurface isolates that most reduced the K_d value for phenanthrene (Table 12), 9711A-2, 9703A-1, 9701A-2, 9702A-2, the rhizosphere isolate A100, and three methanotrophic bacteria, BG8, OB3b, and OBBP were examined further for their mobility through a packed sand column. Fitting of the Cl^- BTCs gave pore water velocities in the column experiments ranging from 0.1 to 0.2 $cm\ min^{-1}$. Results of the BTC for isolate 9711A-2, indicated that this spore-forming, gram (+) bacillus had negligible mobility. In contrast, the other isolates tested appeared to be somewhat mobile. The gram (-) A100 had a length of 1 μm , which, based on consideration of filtration theory¹⁸ and the effect of particle size [McDowell Boyer, 1986 #584], would lend itself to transport. However, only 13 % of the biomass applied to the column was recovered over the duration of the experiment (Figure 21). Although its length was twice that of A100 the mass recovery of the gram (+) bacillus, 9703A-1 was significantly greater (68%) (Figure 22). The mass recovery of isolate 9701A-2, a gram (-) rod, ~1 μm long, was 25% (Figure 23). Compared to the other isolates, 9702A-2, a gram (-) rod, ~1 μm long, was by far the most mobile with a R value of 1.0, and a mass recovery of 86 % (Figure 24). Despite the variable mass recovery, the results of these BTCs demonstrated bacterial mobility. The K_{cell}^s values (based on the R value calculated for the portion of the cell mass recovered in the BTC) of 0.53, 0.65, 0.41, and 0 for strains A100, 9703A-1, 9701A-2 and 9702A-2 respectively, were significantly less than the K_d^s value for phenanthrene with the aquifer sand. These results indicate that cell-bound phenanthrene may be transported at a rate significantly greater than dissolved phenanthrene in soils. The K_{cell}^s values > 0 were indicative of reversible sorption of cells to the sand and slow desorption kinetics was indicated by the tailing of the BTCs¹¹⁹.

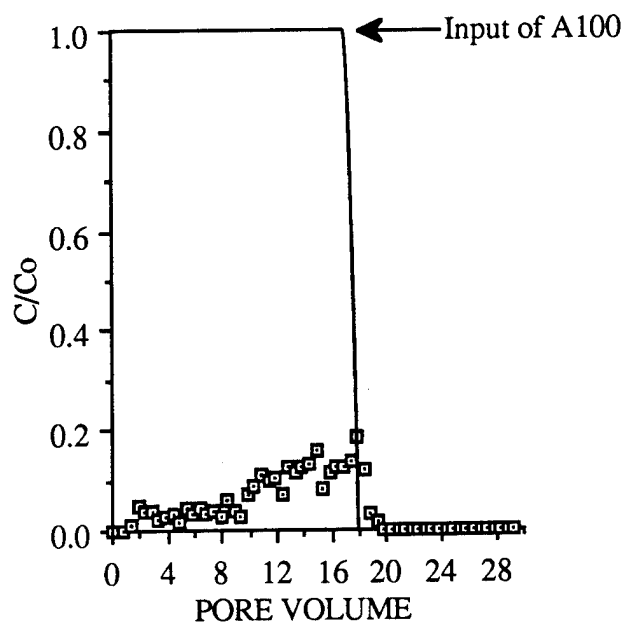


Figure 21. Break-through curve of isolate A100.

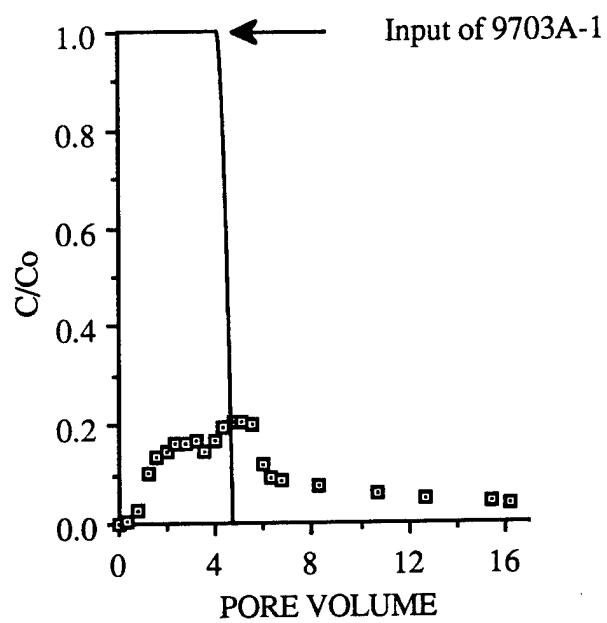


Figure 22. Break-through curve of isolate 9703A-1.

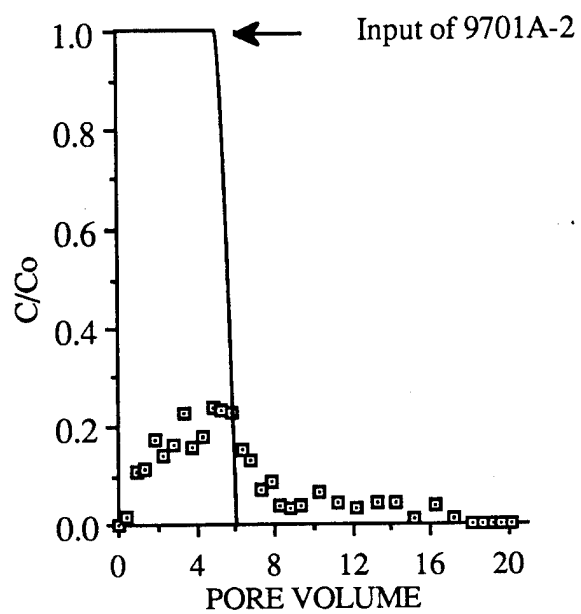


Figure 23. Break-through curve of isolate 9701A-2.

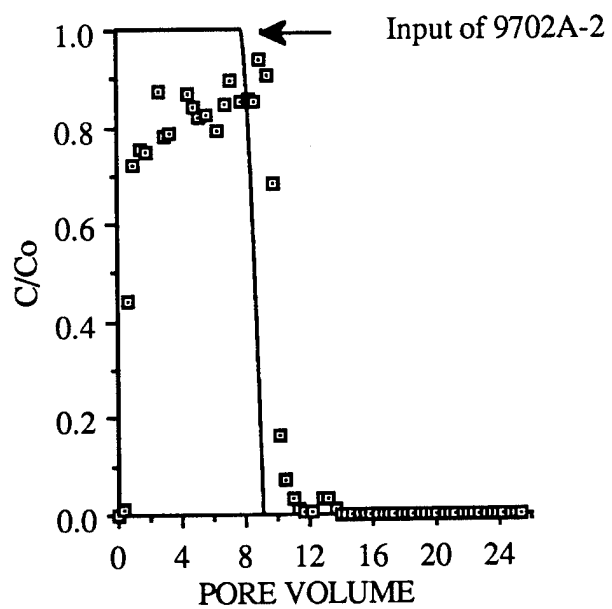


Figure 24. Break-through curve of isolate 9702A-2.

Mobility of the methanotrophic cells was also apparent as seen in their ability to move through a packed column of sand (Figures 25, 26 and 27). Like the subsurface isolates and rhizosphere isolate, mass recovery of the methanotrophic cells was incomplete. Either filtration or irreversible sorption/attachment may account for this phenomenon. The K_d^s for the mobile methanotrophic cells were significantly less than the K_d^s value for phenanthrene with the same aquifer sand (Table 14).

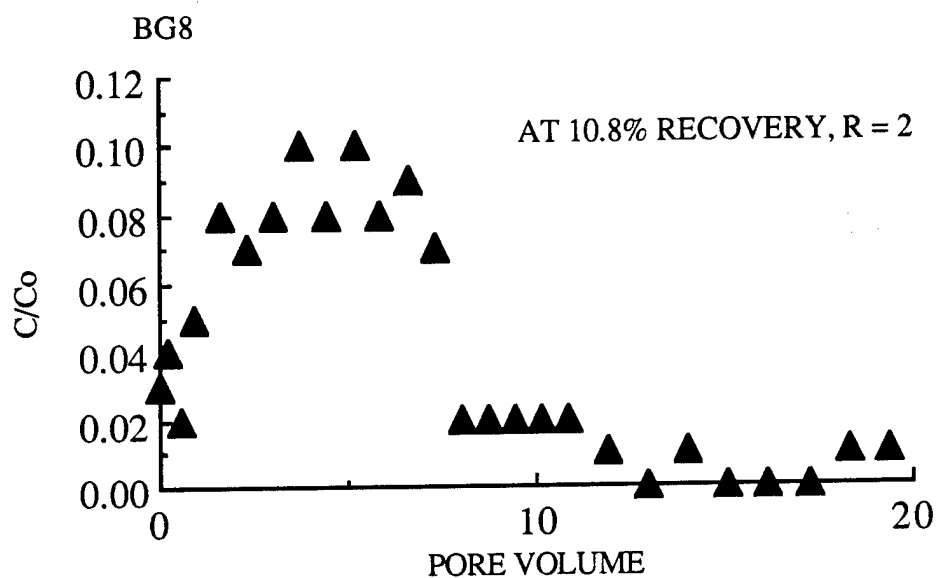


Figure 25. Break-through curve of methanotrophic strain BG8.

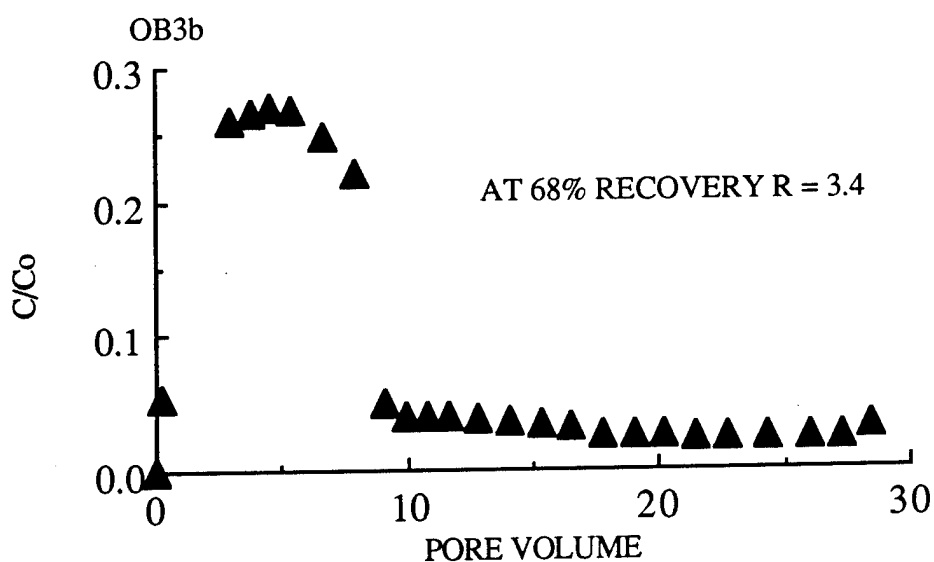


Figure 26. Break-through curve of methanotrophic strain OB3b.

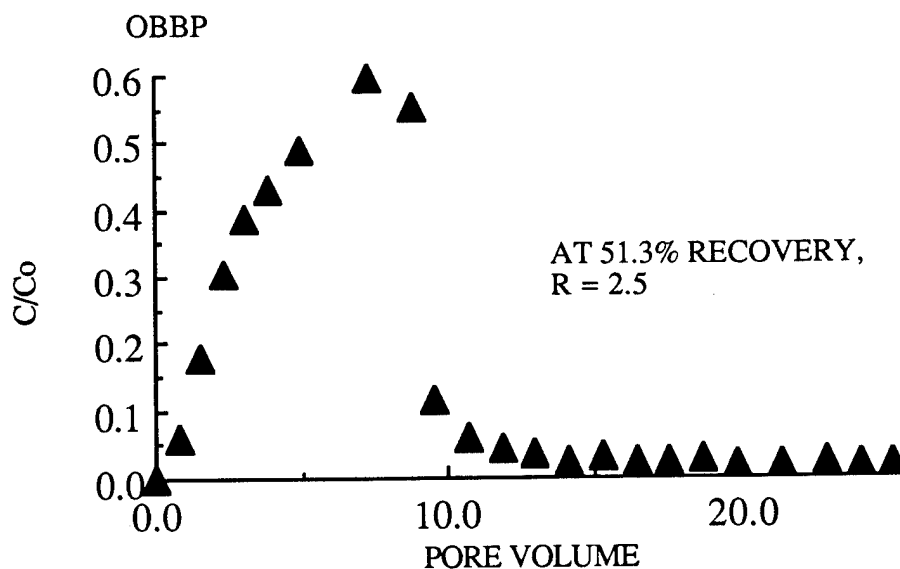


Figure 27. Break-through curve of methanotrophic strain OBBP.

Table 14. Distribution Coefficients For Phenanthrene with Methanotrophic Cells (K_d^{cell}), and for Methanotrophic Cells with an Aquifer Sand (K_{cell}^s).

Bacterium	K_d^{cell} mL/g cell C	K_{cell}^{s*} mL/g
OB3b	16.3×10^3	0.50
OBBP	21.5×10^3	0.34
BG8	11.2×10^3	0.23

*Calculated from R values taken from BTCs using $R = 1 + K_p/n$.

The mobility of a bacterial strain in a porous medium is likely to have a major impact on cells' ability to enhance the transport of a nonpolar compounds. At least two factors may affect the transport or mobility of bacteria through a porous medium: 1. filtration, or physical blockage by pores smaller than the cells ¹²⁰; and 2. sorption, or the reversible attachment/detachment to the surface of the porous medium ¹²¹. The ionic strength of the soil solution may influence both of these phenomena. Some bacterial cells may behave like an inert tracer, and thus have a retardation (R) value of

1. Such was the case with isolate 9702A-2, which appeared to show no indications of sorption to the aquifer sand. The lack of complete mass recovery of this isolate may have been indicative of filtration. In contrast, isolate 9711A-2 was "irreversibly" retained (over the duration of the experiment) by the packed aquifer sand. The incomplete mass recovery of isolates 9703A-1, 9701A-2, A100, and the three methanotrophs was presumed to be the result of either filtration, or sorption, as evident in the tailing of the BTCs for 9703A-1, 9701A-2, BG8, OB3b, and OBBP 23. Lindqvist and Enfield ²³ suggested that irreversible sorption, not physical straining, or filtration was significant in retaining cells. Harvey and Garabedian ¹²² also maintained that straining of their tracer bacteria in field tests was likely to be insignificant because of the relation between the median grain size of the porous material and the dimensions of the bacteria. If the diameter of a colloid is <5% of the respective diameter of the porous medium, straining is generally considered to be insignificant ¹⁷. The cell dimensions of the isolates tested in this research was significantly less than 5% of the the diameters of the particles constituting 95% of the aquifer sand.

The isolates tested for mobility were chosen based on their capacity to reduce the K_d value of phenanthrene for the aquifer sand used in this study. The differences in mobility between the isolates is probably attributable to multiple factors. Gannon et al. ^{123, 124}, for example, observed no correlation between cell surface hydrophobicity, net surface charge, presence of capsular material, or flagella and the variability in transport between several genera of bacteria. They found that apparent filtration of cells was correlated with cell size and dependent on ionic strength. Too few isolates were tested in this study to make meaningful correlations; however, the variable mobility of isolates 9703A-1 and 9711A-2, both of which displayed some hydrophobicity, and were 2x larger than isolates 9701A-2, 9702A-2, and A100, suggest that factors other than cell surface hydrophobicity and size affected bacterial mobility. A significantly greater fraction of 9701A-2 was retained by the aquifer sand than 9702A-2. These differences also suggest that factors other than cell dimension governed retardation or filtration (at constant ionic strength).

L. PHENANTHRENE BTCs WITH AND WITHOUT A BACTERIAL CARRIER

Replicate BTCs indicated that the presence of 65 $\mu\text{g TOC mL}^{-1}$ (equivalent to $\sim 5 \times 10^7$ starved cells mL^{-1}) of isolate 9702A-2 reduced the retardation factor for phenanthrene in the aquifer sand by approximately 25% (Figure 28). The cell concentration employed corresponded to a value on the high end of the natural range of total bacterial densities associated with contaminated subsurface soil environments ⁷³.

These data show that the interaction between a cell's ability to sorb hydrophobic compounds such as phenanthrene and its mobility in porous material can result in enhanced transport of hydrophobic pollutants. Although the distribution coefficient for phenanthrene with isolate 9702A-2 was ~4x less than K_{oc} for phenanthrene with the aquifer sand and 14 % of the cells were retained in the column, the combined effects of phenanthrene sorption to the cells and the mobility of isolate 9702A-2 facilitated the transport of phenanthrene. Recent research by Bellin and Rao ¹¹² indicated that the presence of bacteria in soils may also alter PAH transport by modification of the sorptive properties of the porous medium.

Similarly, using the stainless steel column in place of the all glass column for miscible displacement experiments, transport of phenanthrene in the presence of the mobile type II methanotroph OBBP was significantly enhanced. The BTCs for phenanthrene with and without OBBP (Figure 29) indicated that the presence of 27 μg TOC cells of OBBP mL^{-1} reduced the retardation factor, R , for phenanthrene with the aquifer sand by more than 25%. The reduction of the R value for phenanthrene by the methanotrophic strain OBBP suggests that this type of organism, that was relatively mobile in porous material, and that can be stimulated *in situ* to increase in cell number ¹²⁵, has a demonstrated potential to facilitate transport of PAHs polluting aquifer environments.

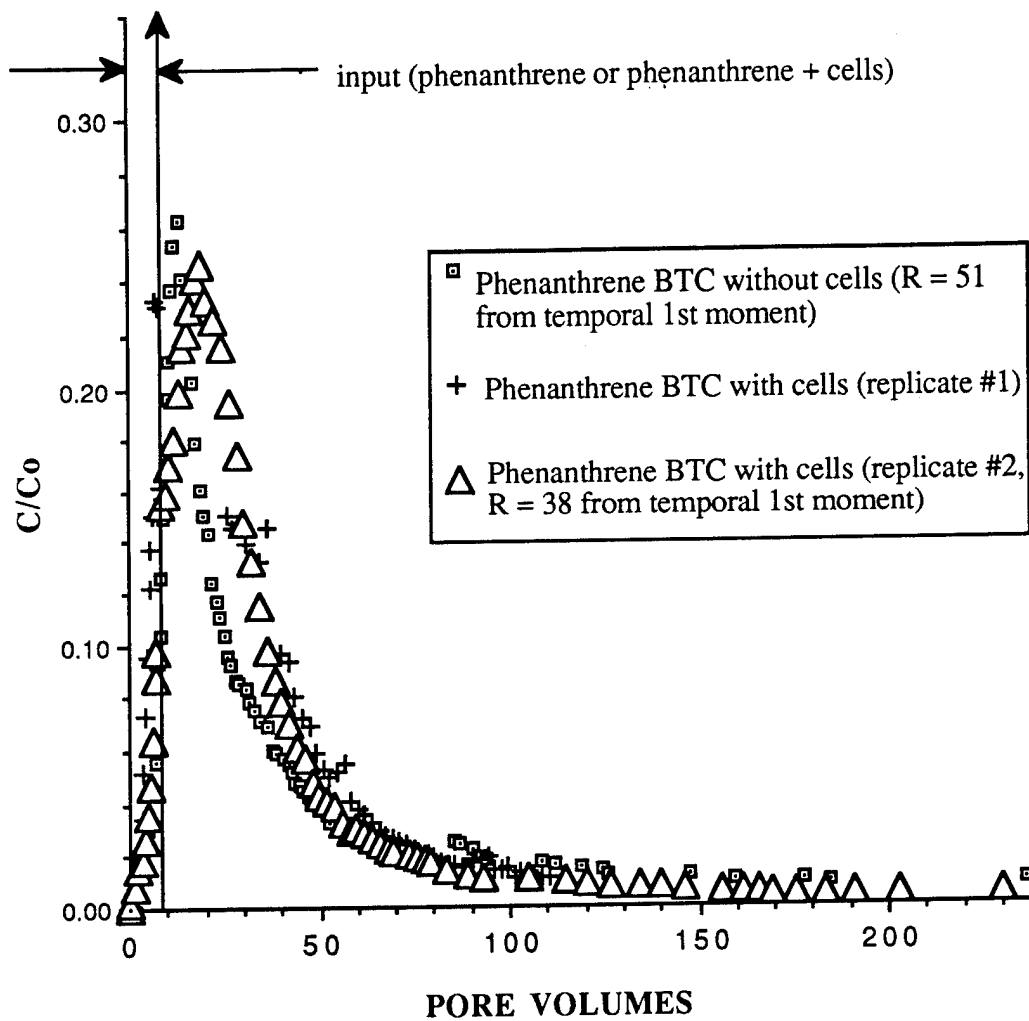


Figure 28. Phenanthrene BTC with and without the presence of the subsurface isolate 9702A-2.

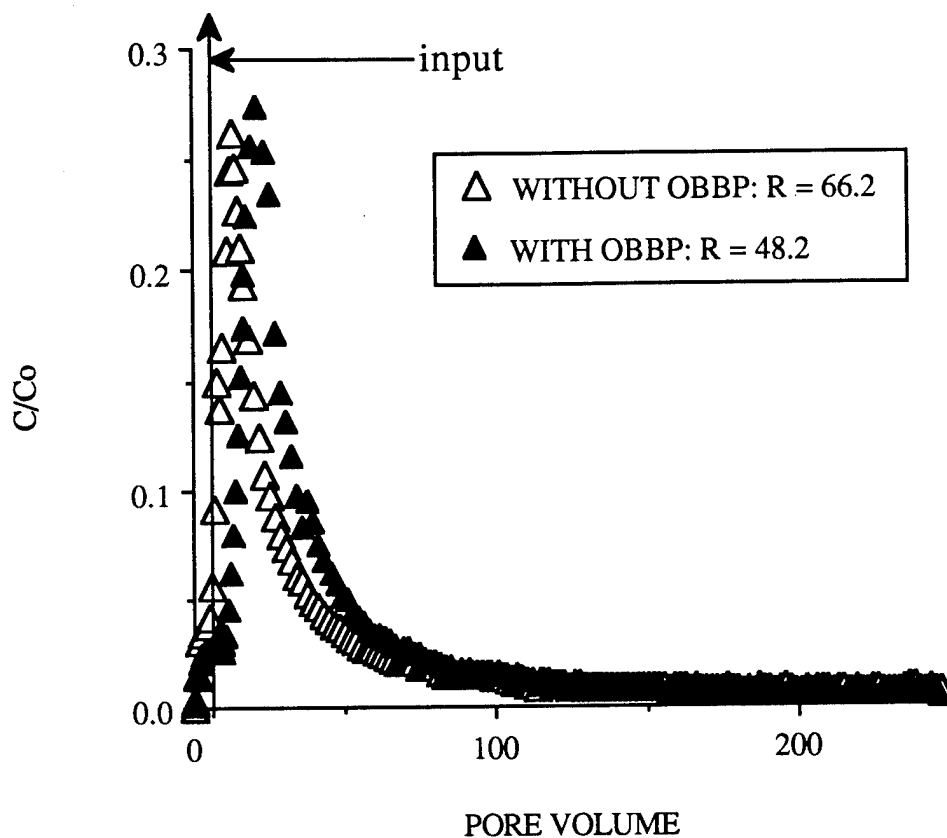


Figure 29. Phenanthrene BTC with and without the presence of the type II methanotroph OBBP

The desorption experiment in which a suspension of 9702A-2 cells followed a pulse of ^{14}C -phenanthrene indicated that a concentration of $57 \mu\text{g TOC cells mL}^{-1}$ reduced the retardation coefficient of phenanthrene with the aquifer sand by 24% (Figure 30). These data support the potential efficacy of mobile cells to facilitate both the desorption and the transport of hydrophobic organic compounds, such as phenanthrene.

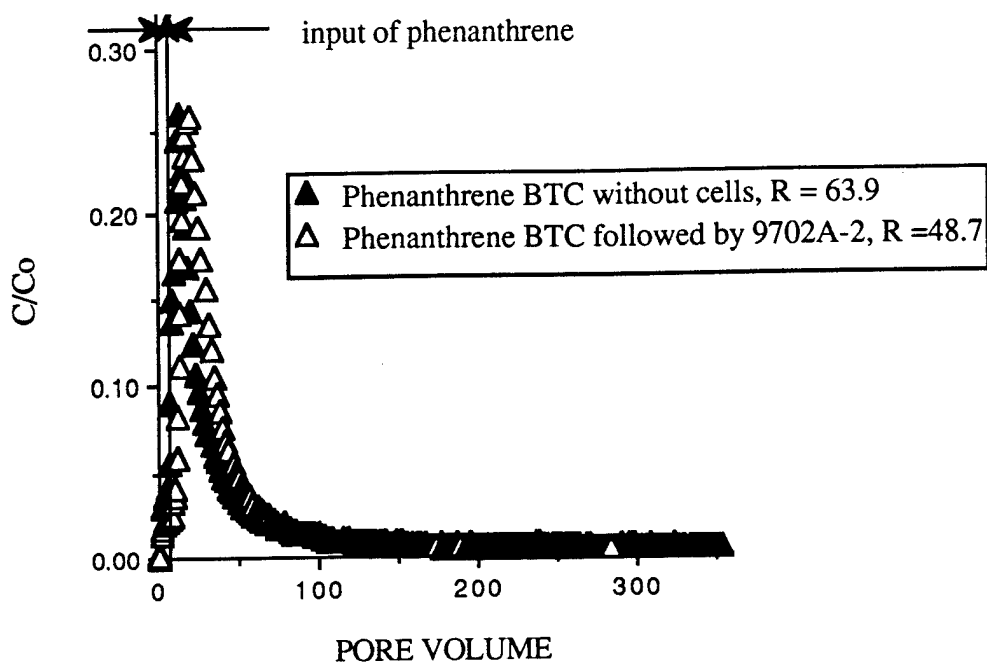


Figure 30. Phenanthrene BTC with and without an eluent containing a cell suspension of isolate 9702A-2 ($57 \mu\text{g TOC mL}^{-1}$).

M. MODEL PREDICTIONS

The predicted reduction in the K_d value for phenanthrene with the subsurface isolate 9702A-2 was compared to observed values (Figure 31). The predictions for enhanced transport of phenanthrene in the presence of isolate 9702A-2, which had a K_{cell}^s value of 0 ($R = 1$), conformed closely with observed values from both the batch and column experiments. Model calculations were also performed to predict the batch results for the bacterial isolates with lower mobility, isolates A100 (Figure 32), and 9703A-1. Model predictions were made for the methanotrophic strains BG8 and OBBP, and were compared to observed reductions in K_d value for phenanthrene with the sand (Figure 33, and 34). Irreversible removal of cells is not considered by the model, and, as anticipated, the model predictions did not compare as closely to observations for these isolates. The observed reduction of K_d for phenanthrene by isolate A100 was between the model calculations in which cells were assumed to be entirely mobile ($K_{\text{cell}}^s = 0$) and reversibly sorbed ($K_{\text{cell}}^s = 0.53$) as was observed in the cell mobility experiment (see Figure 32).

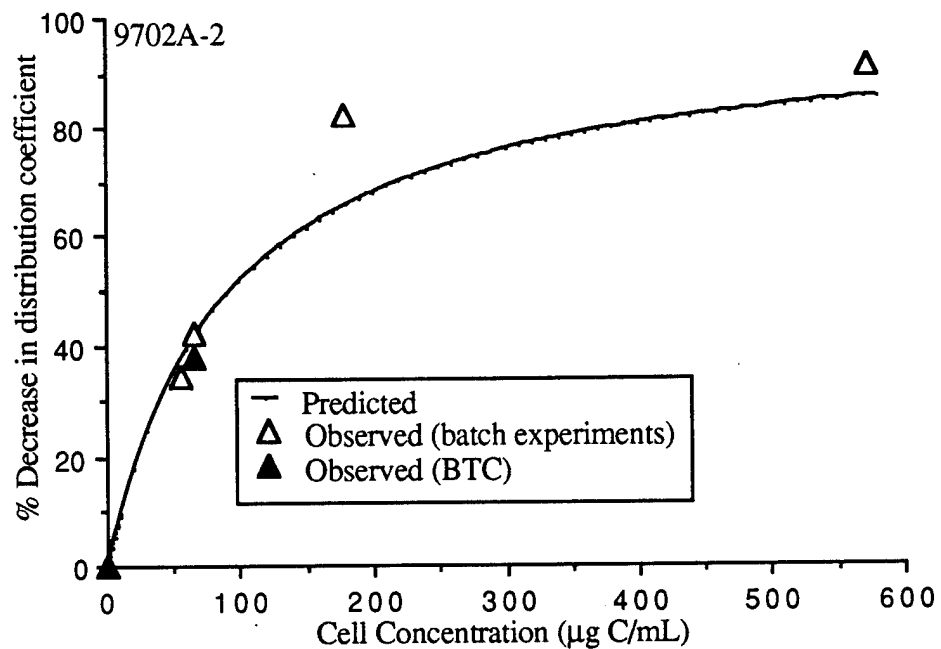


Figure 31. Comparison of predicted and observed reduction of K_d value for phenanthrene with the aquifer sand in the presence of various concentrations of isolate 9702A-2.

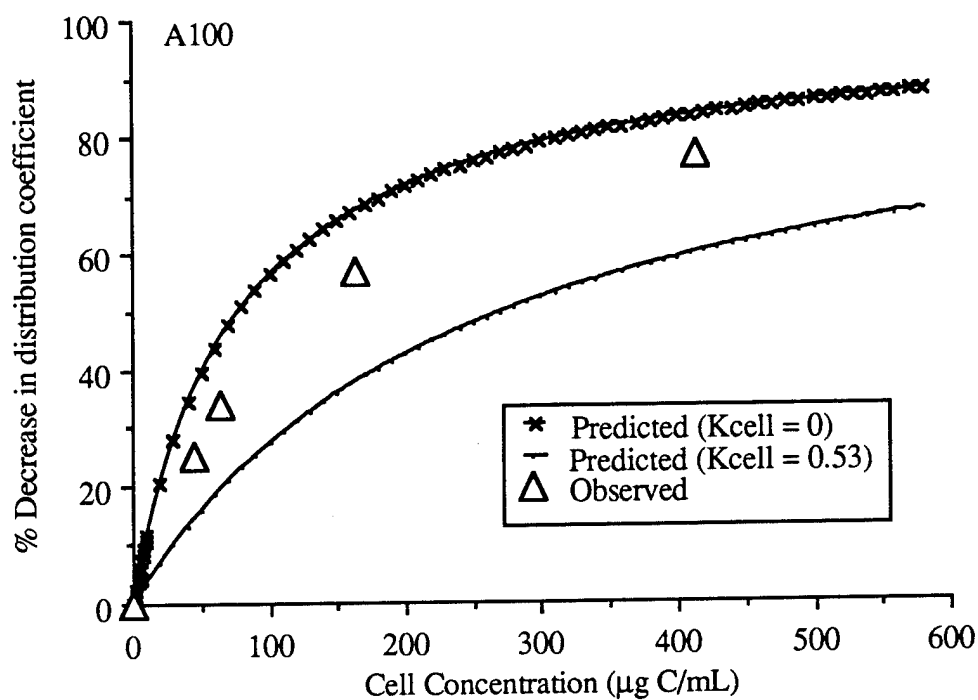


Figure 32. Comparison of predicted and observed reduction of K_d value for phenanthrene with the aquifer sand in the presence of isolate A100.

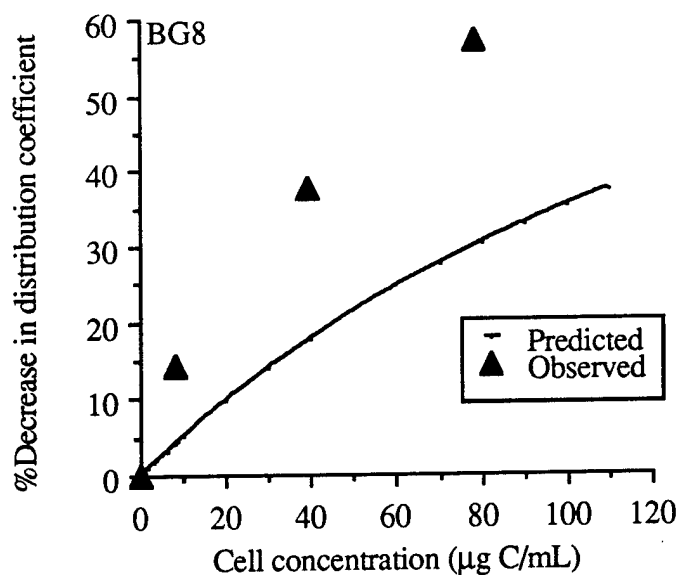


Figure 33. Comparison of predicted and observed reduction of K_d value for phenanthrene with the aquifer sand in the presence of strain BG8.

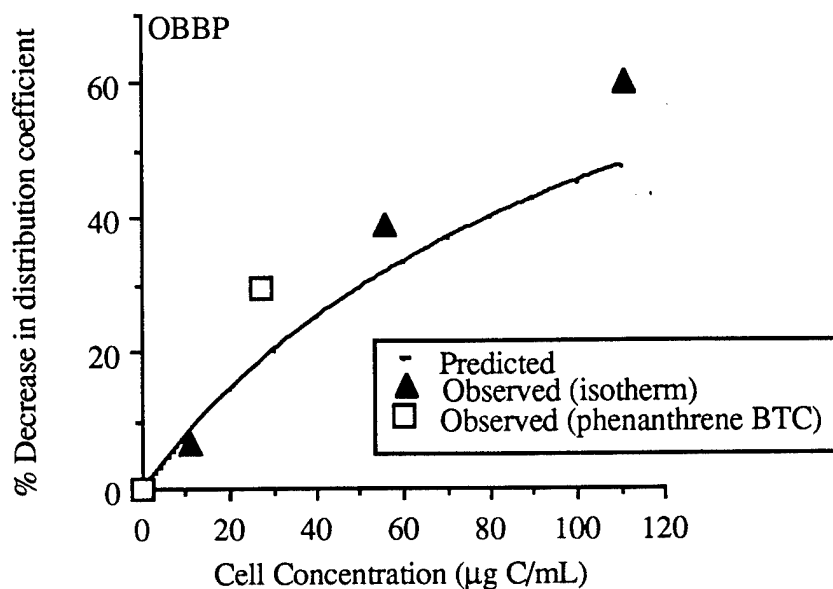


Figure 34. Comparison of predicted and observed reduction of K_d value for phenanthrene with the aquifer sand in the presence of strain OBBP.

The comparison between predicted and observed values in the decrease of K_d value of phenanthrene with the aquifer sand indicated that the three-component model developed by Magee et al. ⁴¹, may be successfully applied to mobile bacterial cells. The calculated reduction in pollutant retardation is related to cell concentration with increased pollutant mobility being anticipated at higher cell concentrations. Engineered forms of aquifer remediation that introduce high concentrations of mobile cells or stimulate their growth *in situ* may merit further research.

N. CULTURE CHARACTERIZATION

1. Bacterial cell hydrophobicity:

Results of the test for microbial adhesion to hydrocarbons (MATH) indicated that *Acinetobacter calcoaceticus* 31012 behaved as it has for others ⁹⁵, i.e., displayed hydrophobic properties. The gram (+) soil isolates, 9703A-1 and 9711A-2, showed moderate hydrophobicity. None of the other isolates selected for testing appeared to possess hydrophobic tendencies (Figure 35). Neither of the naphthalene degrading isolates (Nd9 and N1) appeared to be hydrophobic, suggesting that there may be no physiological connection between cell-surface hydrophobicity and the ability

to degrade sparingly soluble PAHs, or other hydrocarbons as some investigators have suggested ⁶⁰. There also appeared to be no relation between a bacterial cell's ability to sorb phenanthrene and its cell-surface hydrophobicity, as measured by the MATH test. The rhizosphere isolate A100 had a K_d^{cell} value >13,000; while *A. calcoaceticus* had a K_d^{cell} value <6,000.

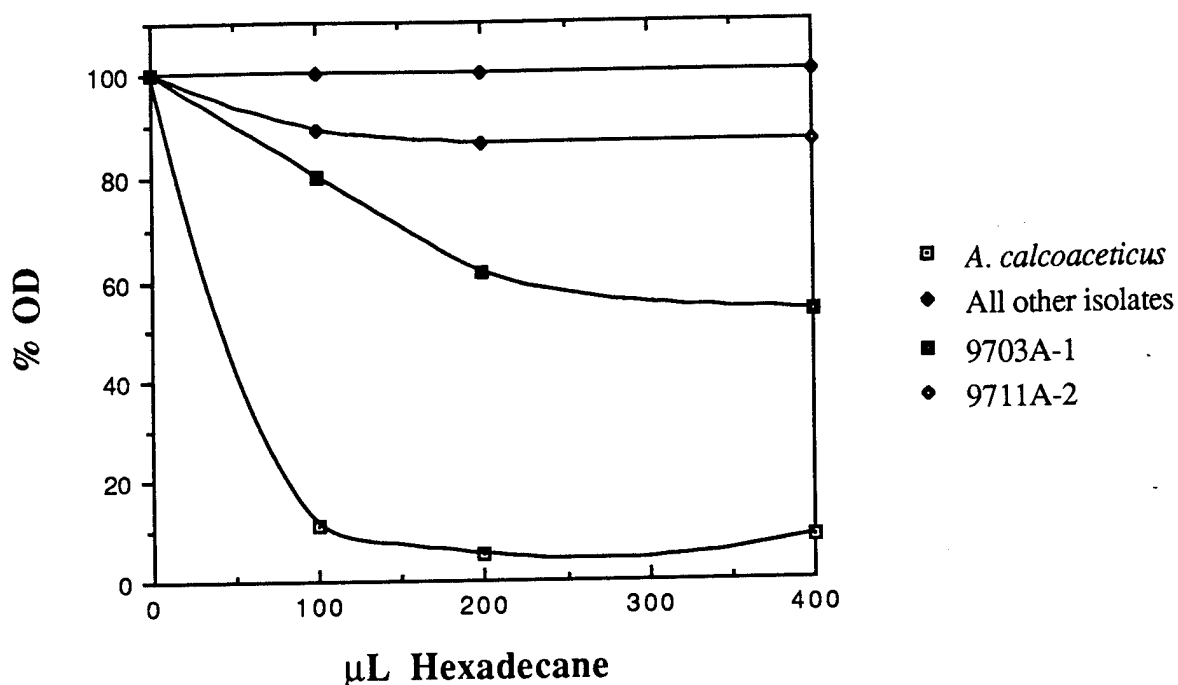


Figure 35. Results of the MATH test showing the characteristic hydrophobicity of *A. calcoaceticus*; the other isolates not showing cell-surface hydrophobicity were 9701A-1, 9702A-2, 9709A-3, 9712M-3, A100, Nd9, and N1.

Based on the MATH test ^{60, 95}, cell surface hydrophobicity appeared not to be a factor in the ability of isolates to sorb phenanthrene. Lindqvist and Enfield ²³ have made similar observations regarding the sorption of HCB and DDT. In fact two of the isolates that most reduced the K_d for phenanthrene with the aquifer sand were less hydrophobic than the *A. calcoaceticus* strain. Many isolates that reduced the K_d for phenanthrene with the aquifer sand to a greater extent than did *A. calcoaceticus* were hydrophilic as defined by the MATH test. Although the reported differences between organic colloids in their ability to sorb the PAH pyrene have been attributed to differences in lipid content or nonpolar character ¹¹⁵, there appeared to be no connection between the affinity of phenanthrene for a bacterial isolate and its cell surface hydrophobicity.

2. Characteristics of isolates 9702M-4 and 9702A-2:

As noted above, soil isolate 9702M-4 produced one of the most effective extracellular polymers in the mobilization of phenanthrene. Soil isolate 9702A-2 was found to be very mobile in the aquifer sand and was an effective colloidal carrier of phenanthrene. Both organisms were gram (-) rods. Results of the transmission electron microscopy showed that both organisms were polarly flagellated. The isolates are, therefore, presumed to be motile, although motility was not observed using light microscopy. The Rapid NFT tests tentatively identified both organisms as *Sphingomonas paucimobilis* (Table 15). Results of the growth rate determinations for isolates 9702M-4 and 9702A-2 indicated that 9702M-4 had a specific growth rate of 0.23/h, or a generation time of 3 h; and 9702A-2 had a specific growth rate of 0.25/h, or generation time of 2.7 h. Neither of the organisms mineralized phenanthrene.

As a result of the characterizations noted above, the isolates 9702M-4 and 9702A-2 were both tentatively designated *Sphingomonas paucimobilis*. Nearly all *Sphingomonas* spp. characterized to date have been clinical isolates associated with various pathologies ¹²⁶. Neither 9702M-4 or 9702A-2 produced yellow-pigmented colonies as do most strains of *S. paucimobilis*. There are also no reports of *S. paucimobilis* being isolated from environmental soil or sediment samples. However, a yellow-pigmented, phenanthrene degrading organism has recently been isolated from an environmental sediment sample from a coal-tar contaminated site (W. Ghiorse, personal communication). Based on API Rapid NFT tests, this organism was also designated a *S. paucimobilis*. It is interesting that these putative *Sphingomonas* strains used in this research were isolated from disturbed sites that were contaminated with PAHs. The isolation of these organisms, and their association with facilitated transport and potential biodegradation may indicate a more widespread distribution of this taxon in the environment than previously recognized. Environmental distribution of *Sphingomonas* spp. appears worthy of further investigation.

Table 15. Rapid NFT Test Results for Isolates 9702M-4 and 9702A-2.

Biochemical/assimilation tests	Isolates	
	9702M-4	9702A-2
Nitrate reduction	-	-
Tryptophanase	-	-
Glucose fermentation	-	-
Arginine dihydrolase	-	-
Urease	+	-
Esculine hydrolysis	+	+
Gelatinase	-	-
Beta-galactosidase	+	+
(assimilations)		
D-Glucose	+	+
L-Arabinose	+	+
D-Mannose	+	+
D-Mannitol	+	+
N-Acetyl-D-glucosamine	+	+
Maltose	+	+
D-Gluconate	-	-
Caprate	-	-
Adipate	-	-
L-Malate	+	+
Citrate	-	-
Phenylacetate	-	-

3. Soluble Methane Monooxygenase Activity of Strain OB3b

Although the methanotrophic strain OB3b expressed soluble methane monooxygenase (sMMO) under low Cu growth conditions, as evidenced by its hydroxylation of naphthalene to form naphthol, the enzyme, under the conditions of the assay, did not hydroxylate phenanthrene to form phenanthrol. The sMMO of *Methylosinus trichosporium* OB3b is well characterized and known to hydroxylate naphthalene. In fact, naphthalene oxidation is a widely used presumptive test for sMMO activity ^{96, 127}. The K_m value of the sMMO from OB3b for naphthalene was

67 μM . The solubility of phenanthrene is $< 6 \times 10^{-3} \mu\text{M}$, which is significantly less than the K_m for naphthalene. The low solubility of phenanthrene may, therefore, account for the negative results of the enzymatic activity of sMMO from strain OB3b on phenanthrene. The ability of the particulate MMO from methanotrophs to transform low concentrations of PAHs such as naphthalene and phenanthrene is poorly characterized. Further work is needed in this area.

SECTION IV

CONCLUSIONS AND RECOMMENDATIONS

The main objective of this research was to test whole bacterial cells and their extracellular polymers for their ability to facilitate the transport of polynuclear aromatic hydrocarbons in porous media. Most prior research on facilitated transport has employed synthetic or exogenic colloids or macromolecules. This research focused on bacterial cells as biocolloids and their extracellular polymers as biogenic macromolecules. Further, the majority of the bacterial strains used in this research were from subsurface microbial populations. The results obtained can be used to help explain observed instances of enhanced transport as well as to modify existing soil remediation technology. The following summary lists the most significant results obtained during the course of this research.

1. The bacterial cells and microbial polymers tested in batch systems were capable of influencing the sorption behavior of phenanthrene onto a low-carbon aquifer sand. Most (70%) of the bacterial isolates decreased the sorption of phenanthrene. While some extracellular polymers actually increased the amount of phenanthrene bound to the sand, most (85%) acted to decrease the amount sorbed. These results demonstrate, that in a static system, microbial polymers and suspended cells can significantly affect the distribution of a PAH onto a low-carbon sorbent.

2. Batch sorption experiments revealed that selected bacterial isolates passively sorbed phenanthrene. Most of the bacterial isolates tested were able to move through packed columns of the low-carbon sand with negligible or slight retardation. Incomplete mass recoveries of the isolates tested indicated varying susceptibility to removal by filtration and/or slow desorption kinetics. The mobility of the cells coupled with their ability to sorb phenanthrene indicated that they have the potential to enhance PAH transport in porous media.

3. The polymer chosen for further study (from soil isolate 9702M-4) had a molecular weight of 500,000 daltons and was mobile in the low-carbon sand. Analysis by fluorescence quenching demonstrated that the polymer was an effective binding agent for phenanthrene. The mobility of extracellular polymers coupled with their ability to sorb phenanthrene indicated that they have the potential to enhance PAH transport in porous media.

4. The efficacy of bacterial extracellular polymers as macromolecular carriers for hydrophobic pollutants was confirmed by miscible displacement experiments in packed columns of the aquifer sand. Column experiments with the extracellular polymer from soil isolate 9702M-4 showed that, at a concentration of 100 mg C/L, the polymer decreased the overall retardation of phenanthrene by 39% (based on 80% recovery of input mass). Phenanthrene peaks appear earlier in the BTC and with higher amplitudes and recoveries than the BTC of phenanthrene in the absence of polymer.

5. The ability of an extracellular polymer to alter phenanthrene sorption onto the sand could not be correlated with the functional groups obtained from IR spectra or molecular size.

6. The presence of extracellular polymer acted to increase the overall mineralization rate of phenanthrene in an aqueous system without a sorbent and did not significantly alter the degradation rate of phenanthrene in an aqueous system with the low-carbon sand sorbent.

7. The efficacy of mobile bacterial cells as colloidal carriers for hydrophobic pollutants was confirmed by miscible displacement experiments in packed columns of the aquifer sand. Column experiments with soil isolate 9702A-2 and a methanotrophic bacterium (OBBP) demonstrated that mobile bacterial cells can decrease the overall retardation of phenanthrene by $\approx 25\%$ at cell concentrations that are within the spectrum that naturally occur in aquifers.

8. The ability of bacteria to sorb phenanthrene and their mobility in the sand bore no apparent relationship to their hydrophobicity as measured by the MATH test.

On the basis of these results, several recommendations for further research are made:

1. Given that certain bacterial cells and extracellular polymers can facilitate transport of the PAH phenanthrene, concerted effort should be made to determine if this facilitated transport can be coupled with enhanced mineralization rates. The bioavailability of sorbed organic pollutants may be limited and agents that enhance pollutant concentration in the aqueous phase have the potential to enhance mineralization rates. Unfortunately, some artificial surfactants inhibit PAH mineralization ⁵⁰. However, dissolved extracellular polymers were demonstrated in this

research to enhance the mineralization of dissolved phenanthrene and did not inhibit phenanthrene mineralization in the presence of a sorbent. Therefore, extracellular polymers may potentially act to enhance both the transport and mineralization of sorbed hydrophobic pollutants. Testing of the coupled processes of PAH mineralization and transport in the presence of extracellular polymers is recommended.

2. Experiments have shown that desorption of phenanthrene previously sorbed to an aquifer sand can be facilitated with cells of soil isolate 9702A-2. The bioavailability of cell-bound phenanthrene was not evaluated in this research; however it is deemed likely that desorption of phenanthrene that is mediated by cells may result in an enhanced rate of degradation. Testing of the coupled processes of PAH mineralization and transport in the presence of mobile cells is recommended.

3. A high fraction of the bacterial strains tested in this research were capable of enhancing PAH transport either by mobility of the cells or by producing mobile extracellular polymers. Two heterotrophic bacteria, 9702M-4 and 9702A-2, have been identified which were particularly effective. Both of these isolates have been tentatively identified as *Sphingomonas paucimobilis*. The presence of this organism at sites of groundwater contamination may serve as an indicator for the processes of enhanced transport. Further characterization of these environmental isolates as well as their polymers should be undertaken. The distribution of this organism, and its association with terrestrial environments contaminated with petroleum residues merits further study.

It must also be recognized that this research considered but a small subset of indigenous soil bacteria and the well-characterized type strains that are known to produce extracellular polymers. Further screening of bacterial isolates and type strains may reveal organisms that are even more effective at enhancing PAH transport than those evaluated in this research. A continuation of the screening process is, therefore, recommended.

4. Results from experiments with methanotrophic bacteria indicated that they have potential to facilitate transport and desorption of PAHs in porous media. In light of these results and published results from field studies demonstrating *in situ* stimulation of methanotrophic populations associated with contaminated groundwater environments 128, 129, further study of these organisms is merited. The possibility exists that *in situ* stimulation of methanotrophs may act to facilitate transport of PAHs as well as their mineralization.

5. Addition of nutrient rich waters to a soil system may cause extensive *in situ* production of polymers. These polymers may remain stationary, which in all likelihood would increase the sorption of hydrophobic pollutants onto the soil due to the increase in organic matter content, or polymers could be released and result in facilitated transport of hydrophobic contaminants. Alternatively polymers may be grown in engineered reactor systems and applied with the infiltrating water in a pump-and treat remediation scheme. In this case, the susceptibility of added polymers to degradation by indigenous soil bacteria would be of concern. Polymer degradation would remove the added carrier from the soil system and could result in biofilm growth and clogging at the injection well. Laboratory experiments are needed to evaluate the feasibility of alternative engineered approaches that are designed to increase polymer concentrations and PAH transport in contaminated porous media. The extent to which microbes can be stimulated to produce polymers *in situ* and the mobility of the polymers that are produced must be evaluated as well as the behavior and fate of added polymers.

Since production of high polymer concentrations *in situ* would require an active microbial population, the effect of these cells in conjunction with polymer production must also be considered. The stimulation of cells that act as mobile colloids that could facilitate pollutant transport in the groundwater but stationary cells may also sorb the pollutant. The utility of selectively stimulating certain bacterial populations (such as methanotrophic bacteria as noted above) merits additional consideration.

6. The research in this report successfully demonstrated the ability of dissolved polymers and mobile bacteria to enhance the transport of a model PAH (phenanthrene) on a low-carbon aquifer sand. The generality of these results for a wide range of pollutants in soils of varying composition must be established to determine the extent to which the composition (of both the sorbent and the sorbate) affects the potential for microbially enhanced transport.

APPENDIX

Under circumstances in which the carrier as well as the pollutant can sorb to the porous medium, the expression for the pollutant's average velocity becomes (a Glossary of is given following the derivation):

$$v_{av} = f_w v_w + f_{om} v_{om} + f_s v_s = f_w v_w + f_{om} v_{om} \quad (A1)$$

The velocity of the stationary phase, v_s is typically assumed equal to zero.

This conceptualization, and the derivation which appears below, is comparable to that employed by West 30. However, an additional relationship is required if the velocity of the carrier has a dissolved and a sorbed component. Assuming the dissolved carrier component has a velocity equal to that of water gives:

$$v_{om} = f_w^{om} v_w + f_s^{om} v_s = f_w^{om} v_w \quad (A2)$$

The term f_w^{om} is defined as:

$$f_w^{om} = \frac{M_w^{om}}{(M_w^{om} + M_s^{om})} \quad (A3)$$

The partition coefficient of the DOM with the stationary phase can be defined as:

$$K_{dom}^s = \frac{M_s^{om} / M_s}{M_w^{om} / V_w} \quad (A4)$$

Solving for M_s^{om} and substituting into Eq. A3 yields:

$$\frac{1}{f_w^{om}} = 1 + \frac{K_{dom}^s M_s}{V_w} = R_{om} \quad (A5)$$

With this expression the average velocity of the organic carrier becomes:

$$v_{om} = f_w^{om} v_w = \frac{v_w}{1 + (K_{dom}^s M_s / V_w)} \quad (A6)$$

and so:

$$R^* = \frac{v_w}{f_w v_w + f_{om} v_{om}} = \frac{1}{f_w + [f_{om} V_w / (1 + K_{dom}^s M_s)]} \quad (A7)$$

The fraction of pollutant which is truly dissolved, f_w , may be defined as:

$$f_w = \frac{M_w^c}{M_w^c + M_{om}^c + M_s^c} \quad (A8)$$

Substitution of the the distribution coefficients for the binding of pollutant to the DOM (K_d^{om}), and to stationary phase, (K_d^s), yields:

$$f_w = \frac{1}{1 + (K_d^{om} M_{om} / V_w) + (K_d^s M_s / V_w)} \quad (A9)$$

Similarly, the fraction of pollutant bound to the DOM is:

$$f_{om} = \frac{K_d^{om} M_{om} / V_w}{1 + (K_d^{om} M_{om} / V_w) + (K_d^s M_s / V_w)} \quad (A10)$$

The expression for the average velocity of the pollutant becomes:

$$v_{av} = f_w v_w + f_{om} v_{om}$$

or

$$v_{av} = \frac{v_w}{1 + (K_d^{om} M_{om} / V_w) + (K_d^s M_s / V_w)} \left[1 + \frac{K_d^{om} M_{om} / V_w}{1 + (K_d^s M_s / V_w)} \right] \quad (A11)$$

Therefore,

$$R^* = \frac{v_w}{v_{av}} = \left(1 + \frac{K_d^{om} M_{om}}{V_w} + \frac{K_d^s M_s}{V_w} \right) \left[\frac{1}{1 + \frac{K_d^{om} M_{om} / V_w}{(1 + K_d^s M_s / V_w)}} \right] \quad (A12)$$

or

$$R^* = \frac{(1 + K_d^{om} \text{DOM} + K_d^s \rho_b / n)}{1 + \frac{K_d^{om} \text{DOM}}{1 + (K_d^s \rho_b / n)}} \quad (A13)$$

A further complication in carrier mediated transport occurs if pore exclusion from small dead-end pores causes the velocity of the carrier (v_{om}^*) to exceed that of the water (v_w). Pore exclusion has been demonstrated for groundwater DOM and a macromolecule (dextran) in soil columns 10, 14. The above model can readily be expanded to incorporate the effect of pore exclusion:

$$v_{om} = f_w^{om} v_{om}^* = \frac{v_{om}^*}{[1 + K_d^s M_s / V_w]} \quad (A14)$$

where v_{om}^* is the velocity of the aqueous organic matter. Therefore,

$$R^{**} = \frac{v_w}{f_w v_w + f_{om} v_{om}} = \frac{1}{f_w + \frac{(v_{om}^* / v_w) f_{om}}{1 + (K_d^s M_s / V_w)}} \quad (A15)$$

The term v_{om}^* / v_w is the extent of enhanced transport of carrier due to pore exclusion; the rest of the terms are as defined in the above derivation of R^* . After substituting for f_w , f_{om} , and M_s / V_w :

$$R^{**} = \frac{1 + K_d^{om} DOM + K_d^s \rho_b / n}{1 + \frac{(v_{om}^* / v_w) K_d^{om} DOM}{1 + K_{dom}^s \rho_b / n}} \quad (A16)$$

As the velocity of the carrier (v_{om}^*) increases, the retardation of the pollutant R^* decreases. If pore exclusion occurs, but is not taken into account, use of the K_{dom}^s from a batch experiment will cause overestimation of carrier retardation, R_{om} , and pollutant retardation, R^* .

Glossary of Symbols

C = concentration in column effluent

C_o = concentration in column influent

DOC = concentration of dissolved organic carbon

DOM = concentration of dissolved organic matter

F = fluorescence intensity in the presence of DOM

F_o = fluorescence intensity in the absence of DOM

f_{oc} = weight fraction of organic carbon in the stationary phase

f_{om} = fraction of solute bound to DOM

f_s = fraction of solute which is sorbed to the stationary phase

f_s^{om} = fraction of DOM which is sorbed to the stationary phase

f_w^{om} = fraction of DOM in the aqueous phase

f_w = fraction of the solute dissolved in water

K_d = distribution coefficient

K_d' = distribution coefficient for the pollutant with the stationary phase in the presence of DOM

K_d^{om} = distribution coefficient for binding of the pollutant to DOM = $\left[\frac{M_{om}^c / M_{om}}{M_w^c / V_w} \right]$

K_d^s = distribution coefficient for the pollutant with the stationary phase = $\left[\frac{M_s^c / M_s}{M_w^c / V_w} \right]$

K_{dom}^s = distribution coefficient for the binding of DOM to the stationary phase

K_{oc} = distribution coefficient on an organic carbon basis = K_d / f_{oc}

$K_{oc}^{om} = K_d^{om}$ on an organic carbon basis

$K_{oc}^s = K_d^s$ on an organic carbon basis

$K_{ocom}^s = K_{dom}^s$ on an organic carbon basis

M_{om} = mass of DOM

M_{om}^c = mass of pollutant associated with DOM

M_s^c = mass of pollutant associated with the stationary phase

M_s = mass of the solid phase

M_w^c = mass of pollutant associated with the aqueous phase

M_s^{om} = mass of DOM associated with the stationary phase

M_w^{om} = mass of DOM associated with the aqueous phase

n = effective porosity of the stationary phase

$[phen]$ = aqueous concentration of free phenanthrene

$[phen \cdot DOM]$ = aqueous concentration of phenanthrene bound to DOM

R = retardation factor

R' = retardation factor for the pollutant in the presence of an unretarded carrier

R^* = retardation factor for the pollutant in the presence of an retarded carrier

R^{**} = retardation factor for the pollutant in the presence of an retarded carrier which undergoes pore exclusion

R_{om} = retardation factor for DOM

V_w = volume of the aqueous phase

v_{av} = average velocity of the pollutant

v_{om} = average velocity of DOM

v_{om}^* = aqueous phase velocity of DOM experiencing pore exclusion

v_s = velocity of the stationary phase

v_w = pore water velocity

ρ_b = bulk density of the stationary phase

ρ_{boc} = bulk density of organic carbon in the stationary phase = $\rho_b f_{oc}$

θ = pore volume

θ_{max} = pore volumes displaced when $C = C_o$

w (as subscript) = aqueous phase

s (as subscript) = stationary phase

o (as subscript) = initial concentration

REFERENCES

1. Smith, J. H., J. C. Harper and H. Jaber, "Analysis and environmental fate of air force distillate and high density fuels," Nat. Tech. Int. Serv., Report No.: ESL-TR-81-54 (1981).
2. Verschueren, K., Handbook of Environmental Data on Organic Chemicals, 2nd Ed., Van Nostrand, NY, (1983).
3. Karickhoff, S. W., D. S. Brown and T. A. Scott, "Sorption of hydrophobic pollutants on natural sediments," Wat. Res., 13, 241-248 (1979).
4. Baker, J. E., P. D. Capel and S. J. Eisenreich, "Influence of colloids on sediment-water partition coefficients of polychlorobiphenyl congeners in natural waters," Environ. Sci. Technol., 20, 1136-1143 (1986).
5. Enfield, C. G., D. M. Walters, R. F. Carsell and S. Z. Cohen, "Approximating transport of organic pollutants to groundwater," Ground Water, 20, 711-722 (1982).
6. Jury, W. A., H. Elabd and M. Resketo, "Field Study of napropamide movement through unsaturated soil," Wat. Resour. Res., 22, 749-755 (1986).
7. Pierce, R. H., C. E. Olney and G. T. Felbeck, "pp'-DDT adsorption to suspended particulated matter in sea water," Geochim. Cosmochim. Acta, 30, 1061-1073 (1974).
8. Vinten, A. J. A., B. Yaron and P. H. Nye, "Verticle transport of pesticides into soil when adsorbed on suspended particles," J. Agric. Food Chem., 31, 662-664 (1983).
9. Brusseau, M. L., A. L. Wood and P. S. C. Rao, "Influence of organic cosolvents on the sorption kinetics of hydrophobic organic chemicals," Environ. Sci. Technol., 25, 903-910 (1991).
10. Enfield, C. G., G. Bengtsson and R. Lindqvist, "Influence of macromolecules on chemical transport," Environ. Sci. Technol., 23, 1278-1286 (1989).
11. Kan, A. T. and M. B. Tomson, "Facilitated transport of naphthalene and phenanthrene in a sandy soil column with dissolved organic matter - macromolecules and micelles," Proceedings of: Petroleum Hydrocarbons and Organic Chemicals in Groundwater, Houston, TX (1986).
12. Magee, B. R., A. T. Lemley and L. W. Lion, "The effect of water-soluble organic material on the transport of phenanthrene in soil," 157-176. In: E. J. Calabrese and P. T. Kostecki (ed.), Petroleum Contaminated Soils, Lewis Publishers, Chelsea, MI (1991).
13. Chudoba, J., J. Hejzlar and M. Dolezal, "Microbial polymers in the aquatic environment-III. Isolation from river, potable and underground water and analysis," Wat. Res., 20, 1223-1227 (1986).
14. Enfield, C. G. and G. Bengtsson, "Macromolecular transport of hydrophobic contaminants in aqueous environments," Ground Water, 25, 64-70 (1988).

15. Harvey, R. W., L. H. George, R. L. Smith and D. R. LeBlanc, "Transport of microspheres and indigenous bacteria through a sandy aquifer: Results of natural and forced gradient tracer experiments," Environ. Sci. Technol., 23, 51 (1989).
16. Hunt, J. R., L. McDowell-Boyer and N. Sitar, "Colloidal migration in porous media: An analysis of mechanics," 453-472. In: (ed.), Coupled Processes Associated with Nuclear Waste Repositories, Academic Press, Inc., New York, NY
17. McDowell-Boyer, L., J. R. Hunt and N. Sitar, "Particle transport through porous media," Wat. Resour. Res., 22, 1901-1921 (1986).
18. Yao, K.-M., M. Y. Habibian and C. R. O'Melia, "Waste and wastewater filtration: Concepts and applications," Environ. Sci. Technol., 5, 1105-1112 (1971).
19. Hagedorn, C., "Microbiological aspects of groundwater pollution due to septic tanks," 181. In: G. Bitton and C. P. Gerba (ed.), Ground Water Pollution Microbiology, John Wiley & Sons, New York, NY (1984).
20. Gerba, C. P., "Phage as indicators of fecal pollution," 197-210. In: S. M. Goyal, C. P. Gerba and G. Bitton (ed.), Phage Ecology, John Wiley and Sons, New York, NY (1987).
21. Baughman, G. L. and D. F. Paris, "Microbial bioconcentration of organic pollutants from aquatic systems--A critical review," Critical Rev. Microbiol., 8, 205-228 (1981).
22. Steen, W. C. and S. W. Karickhoff, "Biosorption of hydrophobic organic pollutants by mixed microbial populations," Chemosphere, 10, 27-32 (1981).
23. Lindqvist, R. and C. G. Enfield, "Biosorption of dichlorodiphenyltrichloroethane and hexachlorobenzene in groundwater and its implications for facilitated transport," Appl. Environ. Microbiol., 58, 2211-2218 (1992).
24. Carter, C. W. and I. H. Suffet, "Binding of DDT to dissolved humic materials," Environ. Sci. Technol., 16, 735-740 (1982).
25. Caron, G., I. H. Suffet and T. Belton, "Effect of dissolved organic carbon on the environmental distribution of non-polar organic compounds," Chemosphere, 14, 993-1003 (1985).
26. Chiou, C. T., R. L. Malcolm, T. I. Brinton and D. E. Kile, "Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids," Environ. Sci. Technol., 20, 502-508 (1986).
27. Chiou, C. T., D. E. Kile, T. I. Brinton, R. L. Malcolm, J. A. Leenheer and P. MacCarthy, "A comparison of water solubility enhancements of organic solutes by aquatic humic materials and commercial humic acids," Environ. Sci. Technol., 21, 1231-1234 (1987).
28. Gamble, D. S., M. I. Haniff and R. H. Zienius, "Solution-phase complexing of atrazine by fulvic acid: A batch ultrafiltration technique," Anal. Chem., 58, 727-731 (1986).

29. Gauthier, T. D., W. R. Seitz and C. L. Grant, "Effects of structural and compositional variations of dissolved humic materials on pyrene K_{oc} values," Environ. Sci. Technol., 21, 243-249 (1987).
30. West, C. C., "Dissolved organic carbon facilitated transport of neutral organic compounds in subsurface systems," Ph.D. thesis, Rice University (1984).
31. Chiou, C. T., L. J. Peters and V. H. Freed, "A physical concept of soil-water equilibria for nonionic organic compounds," Sci., 206, 831-832 (1979).
32. Means, J. C., S. G. Wood, J. J. Hassett and W. L. Banwart, "Sorption of polynuclear aromatic hydrocarbons by sediments and soils," Environ. Sci. Technol., 14, 1524-1528 (1980).
33. Brusseau, M. L. and P. S. C. Rao, "Sorption nonideality during organic contaminant transport in porous media," CRC Crit. Rev. in Environ. Cont., 19, 33-99 (1989).
34. Rutherford, D. W., C. T. Chiou and D. E. Kile, "Influence of soil organic matter composition on the partitioning of organic compounds," Environ. Sci. Technol., 26, 336-340 (1992).
35. Chiou, C. T., "Partition coefficient and water solubility in environmental chemistry," 117-153. In: (ed.), Hazard Assessment of Chemicals, Academic Press, Inc., (1981).
36. Garbarini, D. R. and L. W. Lion, "Influence of the nature of soil organics on the sorption of toluene and trichloroethylene," Environ. Sci. Technol., 20, 1263-1269 (1986).
37. Jardine, P. M., N. L. Weber and J. F. McCarthy, "Mechanisms of dissolved organic carbon adsorption on soil," Soil Sci. Soc. Am. J., 53, 1378-1385 (1989).
38. Stauffer, T. B. and W. G. MacIntyre, "Sorption of low-polarity organic compounds on oxide minerals and aquifer material," Environ. Tox. Chem., 5, 949-955 (1986).
39. Freeze, R. A. and J. A. Cherry, Groundwater, Prentice-Hall, Inc., Englewood Cliffs, NJ (1979).
40. Pankow, J. F. and S. W. McKenzie, "Parameterizing the equilibrium distribution of chemicals between the dissolved, solid particulate matter, and colloidal matter compartments in aqueous systems," Environ. Sci. Technol., 25, 2046-2053 (1991).
41. Magee, B. R., L. W. Lion and A. T. Lemley, "Transport of dissolved organic macromolecules and their effect on the transport of phenanthrene in porous media," Environ. Sci. Technol., 25, 323-331 (1991).
42. Jenkins, M. and L. W. Lion, "Mobile bacteria and transport of polynuclear aromatic hydrocarbons in porous media," Appl. Environ. Microbiol., *in press*, (1993).
43. Gschwend, P. M. and M. D. Reynolds, "Monodisperse ferrons phosphate colloids in an anoxic groundwater plume," J. Cont. Hydro., 1, 309-327 (1987).
44. Means, J. C. and R. Wijayaratne, "Role of natural colloids in the transport of hydrophobic pollutants," Sci., 215, 968-970 (1982).

45. Falatko, D. M. and J. T. Novak, "Effects of biologically produced surfactants on the mobility and biodegradation of petroleum hydrocarbons," Water. Environ. Res., 64, 163-169 (1992).
46. Aronstein, B. N., Y. M. Calvillo and M. Alexander, "Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds," Environ. Sci. Technol., 25, 1728-1731 (1991).
47. Boehm, P. D. and J. G. Quinn, "Solubilization of hydrocarbons by the dissolved organic matter in sea water," Geochim. Cosmochim. Acta, 37, 2459-2477 (1973).
48. Edwards, D. A., R. G. Luthy and Z. Liu, "Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions," Environ. Sci. Technol., 25, 127-133 (1991).
49. Hassett, J. P. and M. A. Anderson, "Association of hydrophobic organic compounds with dissolved organic matter in aquatic systems," Environ. Sci. Technol., 13, 1526-1529 (1979).
50. Laha, S. and R. G. Luthy, "Inhibition of phenanthrene mineralization by nonionic surfactants in soil-water systems," Environ. Sci. Technol., 25, 1920-1930 (1991).
51. Fredrickson, J. K., T. Garland, R. Hicks, J. Thomas, S. Li and K. McFadden, "Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties," Geomicrobiology Journal, 7, 53-66 (1989).
52. Sinclair, J. L. and W. C. Ghiorse, "Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments," Geomicrobiol. J., 7, 15-31 (1989).
53. Costerton, J. W., G. G. Geesey and K.-J. Cheng, "How bacteria stick," Sci. Am., 238, 86-95 (1978).
54. Cooper, D. G. and J. E. Zajic, "Surface-active compounds from microorganisms," Adv. Appl. Microbiol., 26, 229-253 (1980).
55. Singer, M. E., Microbial biosurfactants, (1985).
56. Wilkinson, J. F., "The extracellular polysaccharides of bacteria," Bacteriol. Rev., 22, 46-73 (1958).
57. Sutherland, I. W., Biotechnology of microbial exopolysaccharides, 163, Cambridge University Press, New York (1990).
58. Rosenberg, M. and S. Kjelleberg, "Hydrophobic Interactions: Role in Bacterial Adhesion," In: (ed.), Advances in Microbial Ecology, Plenum Press, New York, NY (1986).
59. Rosenberg, E., A. Zuckerberg, C. Rubinovitz and D. L. Gutnick, "Emulsifier of *Arthobacter* RAG-1: Isolation and emulsifying properties," Appl. Environ. Microbiol., 37, 402-408 (1979).
60. Rosenberg, M., "Basic and applied aspects of microbial adhesion at the hydrocarbon:water interface," CRC Rev. Microbiol., 18, 159-173 (1991).

61. Smith, R. J. and A. G. Collins (ed.). "Microbial enhanced oil recovery: A review," (1985).
62. Rees, D. A., "Stereochemistry and binding behavior of carbohydrate chains," 1-42. In: W. J. Whelan (ed.), Biochemistry of Carbohydrates, University Park Press, Baltimore (1976).
63. Vandevivere, P. and P. Baveye, "Saturated hydraulic conductivity reduction caused by aerobic bacteria in sand columns," Soil Sci. Soc. Am. J., 56, 1-13 (1992).
64. Mian, F. A., T. R. Jarman and R. C. Righelato, "Biosynthesis of exopolysaccharide by *Pseudomonas aeruginosa*," J. Bacteriol., 134, 418-422 (1978).
65. Williams, A. G. and J. W. T. Wimpenny, "Exopolysaccharide production by *Pseudomonas* NCIB 11264 grown in batch culture.," J. Gen. Microbiol., 102, 13-21 (1977).
66. Mitchell, R. and Z. Nevo, "Decomposition of structural polysaccharides of bacteria by marine microorganisms," Nature, 205, 1007-1008 (1965).
67. Pavoni, J. L., M. W. Teeney and W. F. Echelberger Jr., "Bacterial extracellular polymers and biological flocculation," J. Water Poll. Cont. Fed., 44, 414-431 (1972).
68. Martin, J. P. and S. J. Richards, "Decomposition and binding action of a polysaccharide from *Chromobacterium violaceum* in soil," J. Bacteriol., 85, 1288-1294 (1963).
69. Martens, D. A. and W. T. Frankenberger Jr., "Decomposition of bacterial polymers in soil and their influence on soil structure," Biol. and Fertility of Soils, 13, 65-73 (1992).
70. Obayashi, A. W. and A. F. Gaudy Jr., "Aerobic digestion of extracellular microbial polysaccharides," J. Water Poll. Cont. Fed., 45, 1584-1594 (1973).
71. Francis, A. J., C. J. Dodge and J. B. Gillow, "Biodegradation of metal citrate complexes and implications for toxic-metal mobility," Nature, 356, 140-142 (1992).
72. Balkwell, D. L., "Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina," Geomicrobiology Journal, 7, 33-52 (1989).
73. Ghiorse, W. C. and J. T. Wilson, "Microbial ecology of the terrestrial subsurface," Advances in Applied Microbiology, 33, 107-173 (1988).
74. Hagedorn, C., D. T. Hansen and G. H. Simonson, "Survival and movement of fecal indicator bacteria in soil under saturated conditions," J. Environ. Qual., 7, 51-59 (1978).
75. Guerin, W. F. and S. A. Boyd, "Differential bioavailability of soil-sorbed naphthalene to two bacterial species," Appl. Environ. Microbiol., 58, 1142-1152 (1992).
76. Neff, J. M., Polycyclic aromatic hydrocarbons in the aquatic environment, Applied Science Publishers, London (1979).

77. Pankow, J. F. and M. E. Rosen, "Determination of volatile compounds in water by purging directly in a capillary column with whole column cryotrapping," Environ. Sci. Technol., 22, 395-405 (1988).
78. Bulman, T. L. and S. Lesage, "The persistence of polynuclear aromatic hydrocarbons in soil," PACE report #85-2
Petroleum Association for Conservation of Canadian Environment. Ottawa, Ontario., Report No.: (1985).
79. Abdul, A. S., T. L. Gibson and D. N. Rai, "Statistical correlations for predicting the partition coefficient for nonpolar organic contaminants between aquifer organic carbon and water," Haz. Waste & Haz. Mat., 4, 211-221 (1987).
80. Burris, D. R. and W. G. MacIntyre, "Water solubility behavior of binary hydrocarbon mixtures," Envir. Toxicol. Chem., 4, 371-377 (1985).
81. Magee, B. R., "The transport of phenanthrene in soil in the presence of water-soluble organic material," M.S. thesis, Cornell University (1989).
82. Allison, L. E., "Organic carbon," 1367. In: C. A. Black (ed.), Soil Analysis Part 2: Chemical and Microbiological Properties., Amer. Soc. Agronomy, Madison, WI (1965).
83. Hozove, E. C., "Bacterial characteristics important to rhizosphere competence," M.S. Thesis thesis, Cornell University (1989).
84. Madsen, E. L., A. Winding, K. Malachowsky, C. T. Thomas and W. C. Ghiorse, "Contrasts between subsurface microbial communities and their metabolic adaption to polycyclic aromatic hydrocarbons at a forested and an urban coal-tar disposal site," Microb. Ecol., 24, 199-213 (1992).
85. Murgel, G. A., L. W. Lion, C. Acheson, M. L. Shuler, D. Emerson and W. C. Ghiorse, "Experimental apparatus for selection of adherent microorganisms under stringent growth conditions," Appl. Environ. Microbiol., 57, 1987-1996 (1991).
86. Corpe, W. A., "An acid polysaccharide produced by a primary film forming marine bacterium," Devel. Indust. Microbiol., 11, 402 (1970).
87. Kellems, B. L. and L. W. Lion, "Effect of bacterial exopolymer on lead(II) adsorption by $\gamma\text{Al}_2\text{O}_3$ in seawater," Est. Coastal. Shelf Sci., 28, 443-457 (1989).
88. Brown, M. J. and J. N. Lester, "Comparison of bacterial extracellular polymer extraction methods," Appl. Environ. Microbiol., 40, 179-185 (1980).
89. Gauthier, T. D., E. C. Shane, W. F. Guerin, W. R. Seitz and C. L. Grant, "Fluorescence quenching method for determining equilibrium constants for polycyclic aromatic hydrocarbons binding to dissolved humic materials," Environ. Sci. Technol., 20, 1162-1166 (1986).
90. Lion, L. W., T. B. Stauffer and W. G. MacIntyre, "Sorption of hydrophobic compounds on aquifer materials: Analysis methods and the effect of organic carbon," J. Cont. Hydro., 5, 215-234 (1990).

91. Connaughton, D. F., "Sorption kinetics of polynuclear aromatic hydrocarbons on aquifer material: An investigation of long term exposure effects.," M.S. Thesis thesis, Cornell University (1992).
92. Backhus, D. A. and P. M. Gschwend, "Fluorescent polycyclic aromatic hydrocarbons as probes for studying the impact of colloids on pollutant transport in groundwater," Environ. Sci. Technol., 24, 1214-1223 (1990).
93. Parker, J. F. and M. T. van Genuchten, Determining transport parameters from laboratory and field tracer experiments, Virginia Polytechnic Inst. and State University, Blacksburg, VA (1984).
94. Graham, D. W., D. G. Korich, R. P. LeBlanc, N. A. Sinclair and R. G. Arnold, "Applications of a colorimetric plate assay for soluble methane monooxygenase activity," Appl. Environ. Microbiol., 58, 2231-2236 (1992).
95. Rosenberg, M., D. Gutnick and E. Rosenberg, "Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity," FEMS Microbiology Letters, 9, 29-33 (1980).
96. Brusseau, G. A., H.-C. Tsien, R. S. Hanspn and L. W. Wackett, "Optimization of trichloroethylene oxidation by methanotrophs and the use of a colorimetric assay to detect soluble methane monooxygenase activity," Biodegradation, 1, 19-29 (1990).
97. Schwarzenbach, R. P. and J. Westall, "Transport of nonpolar organic compounds from surface water to groundwater: Laboratory sorption studies," Environ. Sci. Technol., 15, 1360-1367 (1981).
98. Stauffer, T. B., W. G. MacIntyre and D. C. Wickman, "Sorption of nonpolar organic chemicals on low carbon content aquifer materials," Environ. Toxicol. Chem., 8, 845 (1989).
99. Gschwend, P. M. and S. Wu, "On the constancy of sediment-water partition coefficients of hydrophobic organic pollutants," Environ. Sci. Technol., 19, 90-96 (1985).
100. Nkedi-Kizza, P., P. S. C. Rao and A. G. Hornsby, "Influence of organic cosolvents on leaching of hydrophobic organic chemicals through soils," Environ. Sci. Technol., 21, 1107-1111 (1987).
101. Valocchi, A. J., "Validity of the local equilibrium assumption for modeling sorbing solute transport through homogeneous soils," Wat. Resour. Res., 21, 808-820 (1985).
102. Dunnivant, F. M., P. M. Jardine, D. L. Taylor and J. F. McCarthy, "Transport of naturally occurring dissolved organic carbon in laboratory columns containing aquifer material," Soil Sci. Soc. Am. J., 56, 437-444 (1992).
103. Jardine, P. M., F. M. Dunnivant, H. M. Selim and J. F. McCarthy, "Comparison of models for describing the transport of dissolved organic carbon in aquifer columns," Soil Sci. Soc. Am. J., 56, 393-401 (1992).

104. Brusseau, M. L. and P. S. C. Rao, "The Influence of sorbate-organic matter interactions on sorption nonequilibrium," Chemosphere, 18, 1691-1706 (1989).
105. Connaughton, D. F., J. Stedinger, L. W. Lion and M. L. Shuler, "Description of Time Varying Desorption Kinetics: Release of Naphthalene from Contaminated Soils," Environ. Sci. Technol., (in press), (1993).
106. Dohse, D. M., "Microbially Facilitated Transport of Polynuclear Aromatic Hydrocarbons in Low-Carbon Aquifer Materials," M.S. Thesis, Cornell University (1992).
107. Pouchert, C., The Aldrich library of infrared spectra, 3, 1870, Aldrich Chemical Company, Milwaukee (1981).
108. Silverstein, R. M., G. C. Bassler and T. C. Morrill, Spectrometric identification of organic compounds, 5th ed., John Wiley & Sons, New York (1991).
109. Hsieh, K. M., "The Growth and Attachment of a Biopolymer Producing *Pseudomonas* Species and the Characterization of its Interactions with a Toxic Trace Metal," PhD thesis, Cornell University (1988).
110. Bodie, E. A., R. D. Schwartz and A. Catena, "Production and characterization of a polymer from *Arthrobacter* species," Appl. Environ. Microbiol., 50, 629-633 (1985).
111. Robinson, J. A., "Determining microbial kinetic parameters using non-linear regression analysis," Advances in Microbial Ecology, 8, 61-114 (1985).
112. Bellin, C. A. and P. S. C. Rao, "Impact of bacterial biomass on contaminant sorption and transport in a subsurface soil," Appl. Environ. Microbiol., 59, 1813-1820 (1993).
113. Chako, C. I. and J. L. Lockwood, "Accumulation of DDT and dieldrin by microorganisms," Canadian Journal of Microbiology, 13, 1123-1126 (1967).
114. Johnson, B. T. and J. O. Kennedy, "Biomagnification of p,p'-DDT and methoxychlor by bacteria," Appl. Environ. Microbiol., 26, 66-71 (1973).
115. Chin, Y.-P. and P. M. Gschwend, "Partitioning of polycyclic aromatic hydrocarbons to marine porewater organic colloids," Environ. Sci. Technol., 26, (1992).
116. McCarthy, J. F. and J. M. Zachara, "Subsurface transport of contaminants," Environ. Sci. Technol., 23, 496-502 (1989).
117. Hou, C. T., A. I. Laskin and P. N. Patel, "Growth and polysaccharide production by *Methylocystis parvus* OBBP on methanol," Appl. Environ. Microbiol., 37, 800-804 (1978).
118. Southgate, G. and P. M. Goodwin, "The regulation of exopolysaccharide production and of enzymes involved in C₁ assimilation in *Methylophilus methylotrophus*," Journal of General Microbiology, 135, 2859-2867 (1989).
119. McCarthy, J. F., T. M. Williams, L. Liang, P. M. Jardine, L. W. Jolley, D. L. Taylor, A. V. Palumbo and L. W. Cooper, "Mobility of natural organic matter in a sandy aquifer," Environ. Sci. Technol., 27, 667-676 (1993).

120. Corapcioglu, M. Y. and A. Haridas, "Transport and fate of microorganisms in porous media," Journal of Hydrology, 72, 149-169 (1984).
121. Marshall, K. C., "Sorptive interactions between soil particles and microorganisms," *In*: A. D. McLaren and J. Skujins (ed.), Soil Biochemistry, Marcel Decker, Inc., New York (1971).
122. Harvey, R. W. and S. P. Garabedian, "Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer," Environ. Sci. Technol., 25, 178-185 (1991).
123. Gannon, J. T., V. B. Manilal and M. Alexander, "Relationship between cell surface properties and transport of bacteria through soil," Appl. Environ. Microbiol., 57, 190-193 (1991).
124. Gannon, J., Y. Tan, P. Baveye and M. Alexander, "Effect of sodium chloride on transport of bacteria in a sodium saturated aquifer material," Appl. Environ. Microbiol., 57, 2497-2501 (1991).
125. Pfiffner, S. M., R. Mackowski, D. C. White and T. J. Phelps, "Monitoring of microbial populations and activities from groundwater for *in-situ* trichloroethylene bioremediation," Proceedings of: American Society for Microbiology 93rd General Meeting-ABSTRACTS, Atlanta, Georgia (1993).
126. Yabuuchi, E., I. Yano, H. Oyaizu, Y. Hashimoto, T. Ezaki and H. Yamamoto, "Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., and two genospecies of the genus *Sphingomonas*," Microbiology and Immunology, 34, 99-119 (1990).
127. Bowman, J. P., L. Jimenez, I. Rosario, T. C. Hazen and G. S. Sayler, "Characterization of the methanotrophic bacterial community present in a trichloroethylene-contaminated subsurface groundwater site," Appl. Environ. Microbiol., 59, 2380-2387 (1993).
128. Semprini, L. P., P. V. Roberts, G. D. Hopkins and P. L. McCarty, "A field evaluation of in situ biodegradation of chlorinated ethenes. II. Results of biostimulation and biotransformation experiments," Ground Water, 28, 715-727 (1990).
129. Bowman, J. P., L. Jimenez, I. Rosario, T. C. Hazen and G. S. Saylor, "Characterization of the methanotrophic bacterial community present in a trichloroethylene-contaminated subsurface groundwater site," Appl. Environ. Microbiol., 59, 2380-2387 (1993).